# PREPARATION AND EVALUATION OF *TRIGONELLA FOENUM GRACEUM* NANOPHYTOSOMES FOR ANTI-DIABETIC ACTIVITY ON WISTAR RATS

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This is certify that the investigation described in this dissertation entitled **"Preparation And Evaluation of *Trigonella Foenum Graceum* Nanophytosomes for Anti-Diabetic Activity on Wistar Rats"**Submitted by Reg.No: 261625354 was carried out in the Department of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126, Which is affiliated to The Tamilnadu Dr.M.G.R.Medical University,Chennai, Under the Guidance of **Dr.V.Lavakumar,M.Pharm.,Ph.D.,** Professor and Research Co-ordinator, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil.

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## EVALUATIONCERTIFICATE

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**Examiners**

1.

2.

DEDICATED TO GOD, MY FAMILY AND MY HUSBAND KARTHIK

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## LIST OF CONTENTS


| S.NO | CHAPTERS | PAGE NO |
|---|---|---|
| 1. | INTRODUCTION | 1 |
| 2. | PLANT PROFILE | 25 |
| 3. | LITERATURE REVIEW | 30 |
| 4. | AIM AND OBJECTIVE | 41 |
| 5. | MATERIALS AND METHODS | 44 |
| 6. | RESULTS AND DISCUSSION | 58 |
| 7. | CONCLUSION | 91 |
| 8. | BIBLIOGRAPHY | 92 |
| 9. | PUBLICATIONS | 100 |

# CHAPTER – I

# INTRODUCTION

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# INTRODUCTION

## 1.1.Diabetes Mellitus

The Diabetes Mellitus is being one of the five leading causes of deaths and debilitating disease in the world. One hundred and fifty million people were suffering from diabetes wide reaching, which is almost five times more than the estimates one decade ago and it may double in the year 2030 [1]. The development of diabetic complications is a major cause of morbidity and mortality and is an ever-increasing burden to healthcare authorities in both developed and developing nations. Epidemiological studies have confirmed that hyperglycemia is the most important factor in the onset and progress of diabetic complications [2].

Diabetes mellitus or simply diabetes is a chronic metabolic disorder of carbohydrate, lipid and protein metabolism characterized by hyperglycemia, glycosurea, hyperlipidemia, negative nitrogen balance and sometimes ketonemia due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action [3]. The hallmark of diabetes mellitus is polyuria-excessive urine production**,** polydipsia-excessive thirst and polyphagia excessive eating [4].

Diabetes is a condition primarily defined by the level of hyperglycaemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications,increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life[5]. The pathogenesis of diabetes mellitus and its complications is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides [6]. However, on chronic usage most of these agents produced several side effects, including hypoglycemic coma, insulin resistance, hyper-sensitivity, cholesterol, jaundice, abdominal pain, anorexia and metallic taste [4].

For various reasons in recent years, the popularity of herbal medicines in diabetic control has increased. Natural plant drugs are frequently considered to be less toxic with lower side effects than synthetic ones [7].Therefore searching herbal product with anti diabetic activity possessing fewer side effects receives considerable publicity and provides an opportunity to cure this disease [8].

## 1.2. CLASSSIFICATION OF DIABETES MELLITUS:

### i. Type 1 diabetes mellitus[9]:

An autoimmune disease in which the immune system mistakenly destroys the insulin-making beta cells of the pancreas. It typically develops more quickly than other forms of diabetes. It is usually diagnosed in children and adolescents, and sometimes in young adults. To survive, patients must administer insulin medication regularly. Type 1diabetes used to be called **juvenile diabetes** and **insulin-dependent diabetes mellitus (IDDM)**. However, those terms are not accurate because children can develop other forms of diabetes, adults sometimes develop type 1, and other forms of diabetes can require insulin therapy. A variation of type 1 that develops later in life, usually after age 30, is called latent autoimmune diabetes of adulthood (LADA). Sometimes patients with
autoimmune diabetes develop insulin resistance because of weight gain or genetic factors. This condition is known as double diabetes.

### ii. Type 2 diabetes mellitus[10]:

A disorder of metabolism, usually involving excess weight and insulin resistance. In these patients, the pancreas makes insulin initially, but the body has trouble using this glucose controlling hormone. Eventually the pancreas cannot produce enough insulin to respond to the body's need for it. Type 2 diabetes is by far the most common form of diabetes, accounting for 85 to 95% of cases in developed nations and an even higher percentage in developing nations, according to the International Diabetes Federation. This disease may take years or decades to develop. It is usually preceded by pre diabetes, in which levels of glucose (blood sugar) are above normal but not high enough yet for a diagnosis of diabetes. People with pre diabetes can often delay or prevent the escalation to type 2 diabetes by losing weight through improvements in exercise and diet, as the Diabetes Prevention Program and other

research projects have demonstrated. Type 2 diabetes used to be called **adult-onset diabetes** and **non-insulin-dependent diabetes mellitus (NIDDM)**. Those terms are not accurate because children can also develop this disease, and some patients require insulin therapy.

**iii. Other specific type (Monogenic diabetes)[10]:**

Diabetes caused by another condition. The many potential sources of secondary diabetes range from diseases such as pancreatitis, cystic fibrosis, Down syndrome and hemochromatosis to medical treatments including corticosteroids, other immunosuppressives, diuretics and pancreatectomy.

**iv. Gestational Diabetes Mellitus (GDM)[11]:**

A temporary metabolic disorder that any previously nondiabetic woman can develop during pregnancy, usually the third trimester. Hormonal changes contribute to this disease, along with excess weight and family history of diabetes. About 4% of pregnant women develop gestational diabetes, according to the American Diabetes Association.

| Blood Sugar Classification | Fasting Blood Sugar Levels | Post Meal Blood Sugar Levels |
|---|---|---|
| Normal | 70-100 mg/dL | 70-140 mg/dL |
| Prediabetes | 101-125 mg/dL | 141-200 mg/dL |
| Diabetes | 125 mg/dL and above | 200 mg/dL and above |

**Figure 1: Blood sugar levels**

## 1.3.INSULIN BIOSYNTHESIS, SECRETION, AND ACTION [12]

### Biosynthesis

Insulin is produced in the beta cells of the pancreatic islets. It is initially synthesized as a single chain 86-amino-acid precursor polypeptide, preproinsulin. Subsequent proteolytic processing removes the aminoterminal signal peptide, giving rise to proinsulin. Proinsulin is structurally related to insulin-like growth factors I and II, which bind weakly to the insulin receptor. Cleavage of an internal 31-residue fragment from proinsulin generates the C peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulfide bonds. The mature insulin molecule and C peptide are stored together and co-secreted from secretory granules in the beta cells. Because C peptide is cleared more slowly than insulin, it is a useful marker of insulin secretion and allows discrimination of endogenous and exogenous sources of insulin in the evaluation of hypoglycemia. Pancreatic beta cells co-secrete islet amyloid polypeptide (IAPP) or amylin, a 37-amino-acid peptide, along with insulin. The role of IAPP in normal physiology is incompletely defined, but it is the major component of the amyloid fibrils found in the islets of patients with type 2 diabetes, and an analogue is sometimes used in treating type 1 and type 2 DM. Human insulin is produced by recombinant DNA technology; structural alterations at one or more amino acid residues modify its physical and pharmacologic characteristics.

### Secretion

Glucose is the key regulator of insulin secretion by the pancreatic beta cell, although amino acids, ketones, various nutrients, gastrointestinal peptides, and neurotransmitters also influence insulin secretion. Glucose levels >3.9 mmol/L (70 mg/dL) stimulate insulin synthesis, primarily by enhancing protein translation and processing. Glucose stimulation of insulin secretion begins with its transport into the beta cell by a facilitative glucose transporter **(Fig. 2)**. Glucose phosphorylation by glucokinase is the rate-limiting step that controls glucose-regulated insulin secretion. Further metabolism of glucose-6-phosphate via glycolysis generates ATP, which inhibits the activity of an ATP-sensitive K+ channel. This channel consists of two separate proteins: one is the binding site for certain oral hypoglycemics (e.g., sulfonyl-ureas, meglitinides); the other is an inwardly rectifying K+ channel protein

(Kir6.2). Inhibition of this K+ channel induces beta cell membrane depolarization, which opens voltage-dependent calcium channels (leading to an influx of calcium), and stimulates insulin secretion. Insulin secretory profiles reveal a pulsatile pattern of hormone release, with small secretory bursts occurring about every 10 min, superimposed upon greater amplitude oscillations of about 80–150 min. Incretins are released from neuroendocrine cells of the gastrointestinal tract following food ingestion and amplify glucose stimulated insulin secretion and suppress glucagon secretion. Glucagon-like peptide 1 (GLP-1), the most potent incretin, is released from L cells in the small intestine and stimulates insulin secretion only when the blood glucose is above the fasting level. Incretin analogues, are used to enhance endogenous insulin secretion.

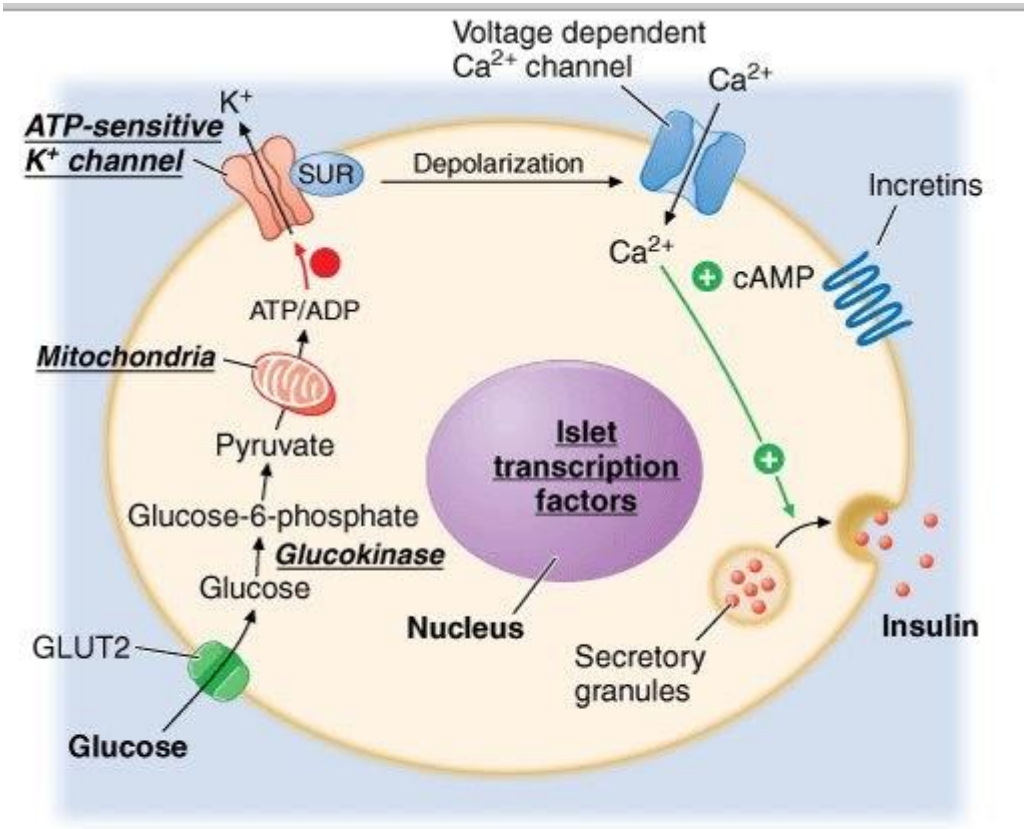




**Figure 2 Glucose mediated insulin secretion**

**Action**

Once insulin is secreted into the portal venous system, 50% is removed and degradedby the liver. Unextracted insulin enters the systemic circulation where it binds to receptors in target sites. Insulin binding to its receptor stimulates intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and the recruitment of intracellular signaling molecules, such as insulin receptor substrates (IRS) **(Fig. 3)**. IRS and other adaptor proteins initiate a complex cascade of phosphorylation and dephosphorylation reactions, resulting in the widespread metabolic and mitogenic effects of insulin. As an example, activation of the phosphatidylinositol-3′-kinase (PI-3-kinase) pathway stimulates translocation of a facilitative glucose transporter (e.g., GLUT4) to the cell surface, an event that is crucial for glucose uptake by skeletal muscle and fat. Activation of other insulin receptor signaling pathways induces glycogen synthesis, protein synthesis, lipogenesis, and regulation of various genes.

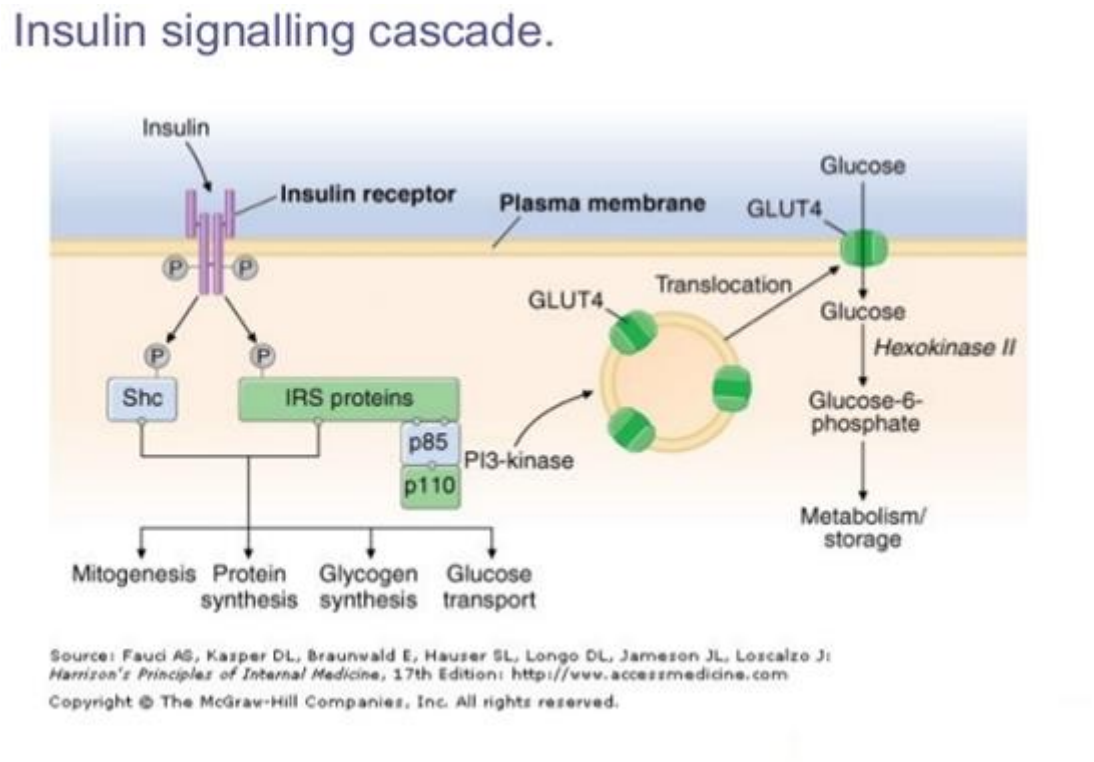


**Figure 3 Insulin signaling cascade**

Glucose homeostasis reflects a balance between hepatic glucose production and peripheral glucose uptake and utilization. Insulin is the most important regulator of this metabolic equilibrium, but neural input, metabolic signals, and other hormones

(e.g., glucagon) result in integrated control of glucose supply and utilization. In the fasting state, low insulin levels increase glucose production by promoting hepatic gluconeogenesis and glycogenolysis and reduce glucose uptake in insulin sensitive tissues (skeletal muscle and fat), thereby promoting mobilization of stored precursors such as amino acids and free fatty acids (lipolysis). Glucagon, secreted by pancreatic alpha cells when blood glucose or insulin levels are low, stimulates glycogenolysis and gluconeogenesis by the liver and renal medulla. Postprandially, the glucose load elicits a rise in insulin and fall in glucagon, leading to a reversal of these processes. Insulin, an anabolic hormone, promotes the storage of carbohydrate and fat and protein synthesis. The major portion of postprandial glucose is utilized by skeletal muscle, an effect of insulin-stimulated glucose uptake. Other tissues, most notably the brain, utilize glucose in an insulin-independent fashion. [12]

### 1.4.SIGNS AND SYMPTOMS

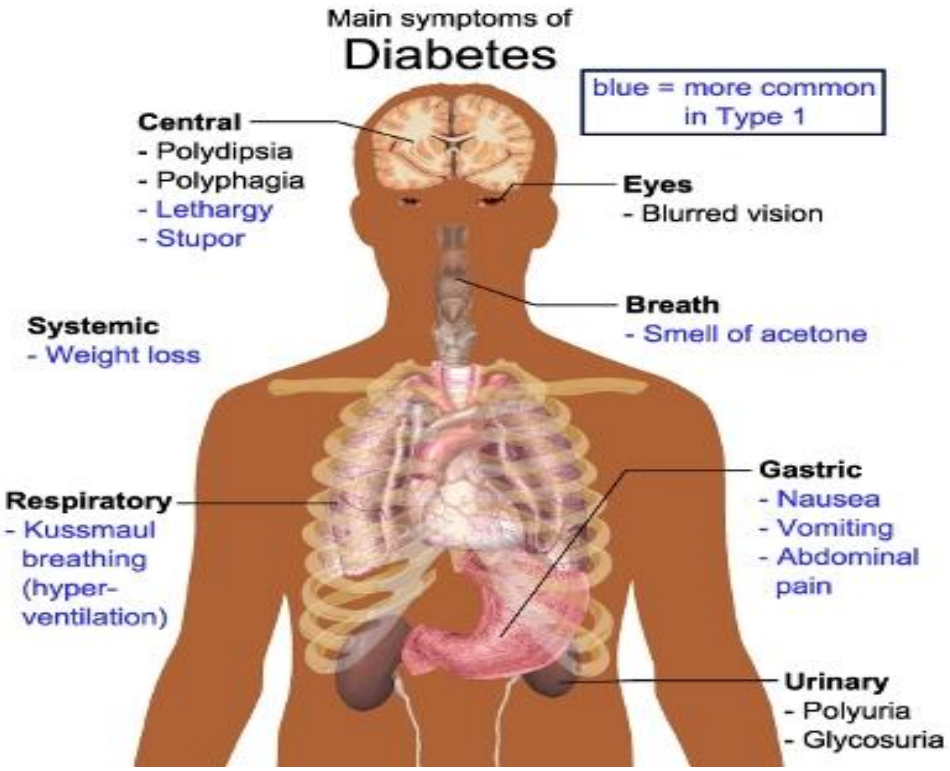


**Figure 4: Signs and symptoms of diabetes**

**1.5.RISK FACTORS**: [37]

- Obesity
- Age (onset of puberty is associated with increased insulin resistance)
- Lack of physical activity
- Genetic predisposition
- Racial/ethnic background (African American, Native American, Hispanic and Asian/Pacific Islander)
- Conditions associated with insulin resistance, (e.g., polycystic ovary syndrome).
- Family history of T2DM in first- or second-degree relative[13]
- High-calorie diet

**1.5.1.COMPLICATIONS OF DIABETES MELLITUS** [13]

**I. Acute complications**

- Hypoglycemia
- Hyperglycemic crises
    a. Diabetes Ketoacidosis (DKA)
    b. Hyperglycemic hyperosmolar state (HHS)

**II. Chronic complications:**

- Micro vascular complications
    a. Diabetic retinopathy
    b. Diabetic nephropathy
    c. Diabetic neuropathy
- Macro vascular disease

**III. Other complications and associated conditions**

- Impaired growth and development
- Associated autoimmune conditions
    a. Hypothyroidism
    b. Hyperthyroidism
    c. Celiac disease
    d. Vitiligo
    e. Primary adrenal insufficiency (Addison's disease)
- Lipodystrophy (lipoatrophy and lipohypertrophy)

- Necrobiosis lipoidica diabeticorum
- Non-alcoholic fatty liver disease
- Infections seen in patients with diabetes
- Limited joint mobility
- Edema

**Diabetes Ketoacidosis (DKA)**

DKA results from relative or absolute insulin deficiency combined with counter regulatory hormone excess (glucagon, catecholamine, cortisol, and growth hormone). Both insulin deficiency and glucagon excess, in particular, are necessary for DKA to develop. The decreased ratio of insulin to glucagon promotes gluconeogenesis, glycogenolysis, and ketone body formation in the liver, as well as increases in substrate delivery from fat and muscle (free fatty acids, amino acids) to the liver.

Nausea and vomiting, abdominal pain which resemble acute pancreatitis or ruptured viscus, Kussmaul respirations and a fruity odor on the patient's breath (secondary to metabolic acidosis and increased acetone) are classic signs of the disorder. [14]

**Hyperglycemic hyperosmolar state (HHS)**

The prototypical patient with HHS is an elderly individual with type 2 DM, with a several-week history of polyuria, weight loss, and diminished oral intake that culminates in mental confusion, lethargy, or coma. The physical examination reflects profound dehydration and hyper osmolality and reveals hypotension, tachycardia, and altered mental status. Notably absent are symptoms of nausea, vomiting, and abdominal pain and the Kussmaul respirations characteristic of DKA. [14]

**Chronic Complications**

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications.

The vascular complications of DM are further subdivided into micro vascular (retinopathy,neuropathy, and nephropathy) and macro vascular complications [coronary heart disease (CHD), peripheral arterial disease (PAD), cerebrovascular disease].

Nonvascular complications include problems such as gastroparesis, infections, and skin changes. Long-standing diabetes may be associated with hearing loss. [15]

**Diabetic retinopathy**

DM is the leading cause of blindness. Blindness is primarily the result of progressive diabetic retinopathy and clinically significant macular edema. Diabetic retinopathy is classified into two stages: non proliferative and proliferative.

Non proliferative diabetic retinopathy usually appears late in the first decade or early in the second decade of the disease and is marked by retinal vascular micro aneurysms, blot hemorrhages, and cotton-wool spots. Mild non proliferative retinopathy progresses to more extensive disease, characterized by changes in venous vessel caliber, intraretinal micro vascular abnormalities, and more numerous microaneurysms and hemorrhages. [15]

**Necrobiosis lipoidica diabeticorum**

It is a rare disorder of DM that predominantly affects young women with type 1 DM, neuropathy, and retinopathy. It usually begins in the pretibial region as an erythematous plaque or papules that gradually enlarge, darken, and develop irregular margins, with atrophic centers and central ulceration. They may be painful. [15]

**Lipoatrophy and lipohypertrophy**

It occur at insulin injection sites but are now unusual with the use of human insulin. Xerosis and pruritus are common and are relieved by skin moisturizers. [15]

## 1.6. DIAGNOSIS OF DIABETES MELLITUS

**Random plasma test**

• The simplest test and doesn't require fasting before taking the test.

• If 200 or more than 200 mg/dl of blood glucose it probably indicates diabetes but has to be reconfirmed.

**Fasting plasma glucose test:**

• There should be eight hours fasting before taking this test. Blood glucose more than 126 mg/dl on two or more tests conducted on different days confirms a diabetes diagnosis [16].

**Oral glucose tolerance test**

• When random plasma glucose test is 160-200 mg/dl and the fasting plasma test is 110-125 mg/dl, then this test is conducted [11].

• This blood test evaluates body's response to glucose. This test requires fasting at least eight but not more than 16 hrs.

• Fasting glucose level is determined, and then gives 75 gm of glucose, 100 gm for pregnant women. The blood is tested every 30 minutes to one hr for two or three hrs.

• This test is normal if your glucose level at two hrs is less than 140 mg/dl. A fasting level of 126 mg/dl or greater and two hour glucose level of 200 mg/dl or higher confirms a diabetes diagnosis[16].

## 1.7. MANAGEMENT OF DIABETES

### 1.7.1. Oral hypoglycemic agents:

Oral hypoglycemic agents are used in the treatment of **type 2** diabetes mellitus. The classification of these drugs are given below

**Table 1: Represents list of oral hypoglycemic agents used for the therapy of diabetes mellitus[17-19]**

| S. No | Class of Drug | Examples | Mechanism of Action | Adverse Effects Listed |
|---|---|---|---|---|
| 1. | Biguanides | Metformin<br>Phenformin | Supress Gluconeogenesis and glucose output from liver. | Abdominal pain,Anorexia,Mild diarrhea,Metallic taste,Lactic acidosis. |
| 2. | Sulfonylureas | **1st generation:**<br><br>Tolbutamide<br>Chlorpropamide<br>**2nd generation:** | It causes depolarization of the β cell membrane and thus it enhance calcium influx which in turn increase the | Hypoglycemia,Nausea, vomiting,headache, photosensitivity,rarely agranulocytosis may occur. |

| | | | | |
|---|---|---|---|---|
| | | Metformin<br>Glipizide<br>Glyclazide<br>Glimipride | release of insulin from β cells. | |
| 3. | Meglitinide analogues | Repaglinide<br>Nateglinide | Bind to receptors and closure of ATP dependent K* channels causes depolarization and promote insulin release. | Dizzness,Dyspepsia ,Arthalgia,Flu like symptoms may occur. |
| 4. | Thiazolidones | Pioglitazone<br>Rosiglitazone | Activation of genes regulating fatty acid metabolism and lipogenesis in adipose tissue contributes to the insulin sensitizing action. | Mild Anemia,Plasma volume expansion,Edema, weight gain, head ache. |
| 5. | α Glucosidase inhibitors | Acarbose<br>Miglitol | Inhibit α glucosidase enzyme ,there by it slows down the digestion of carbohydrates. | Flatulence,Abdominal discomfort,Loose stools. |

### 1.7.2. Insulin derivatives:

Insulin is a harmone secreted from the beta cells of pancreas which regulate the blood glucose level in the body.Insufficient insulin secretion leads to diabetes mellitus. Insulin derivatives are mainly used in the treatment of **type-1** diabetes.To

maintain the insulin level, some synthetic insulin derivatives are synthesized and their classes are given below

**Table 2: Represents list of insulin derivatives used for therapy of diabetes mellitus[20-22]**

| S.NO | TYPE OF INSULIN | APPEARANCE | ONSET OF ACTION | DURATION OF ACTION |
|---|---|---|---|---|
| 1. | **Rapid acting:** Insulin lispro Insulin aspart | Clear | 0.2-0.4 hr | 3-5hr |
| 2. | **Short acting:** Clear (soluble) insulin | Clear | 0.5-1hr | 6-8hr |
| 3. | **Intermediate acting:** Insulin zinc suspension or lente Isophane insulin | Cloudy | 1-2hr | 20-24hr |
| 4. | **Long acting:** Protamine zinc insulin Insulin glargine | Cloudy | 2-4hr | 24-36 hr |

## 1.8. INTRODUCTION OF MEDICINAL PLANTS

Medicinal plants play an important role in the development of potent therapeutic agents. Today estimate that about 80 % of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. Herbal drugs referred as plants materials or herbals, involves the use of whole plants or parts of plants, to treat injuries or illnesses[23]. Herbal drugs are use of therapeutic herbs to prevent and treat diseases and ailments or to support health and healing. These are drugs or preparations made from a plant or plants and used for any of such purposes. Herbal drugs are the oldest form of health care known to

mankind. World Health Organization (WHO) has distinct herbal drugs as complete, labelled medicinal products that have vigorous ingredients, aerial or secretive parts of the plant or other plant material or combinations. World Health Organization has set precise guidelines for the evaluation of the safety, efficacy, and quality of herbal medicines. Herbal drug is a chief constituent in traditional medicine and a common constituent in ayurvedic, homeopathic, naturopathic and other medicine systems. Herbs are usually considered as safe since they belong to natural sources. The use of herbal drugs due to toxicity and side effects of allopathic medicines, has led to rapid increase in the number of herbal drug manufacturers. For the past few decades, herbal drugs have been more and more consumed by the people with no prescription. These drugs have survived real world testing and thousands of years of human testing. Some drugs have been discontinued due to their toxicity, while others have been modified or combined with additional herbs to counterbalance side effects[24].

### 1.8.1.Advantages of Herbal Drugs

1. High Low/Minimum cost
2. Complete accessibility
3. Enhanced tolerance
4. More protection
5. Fewer side-effects
6. Potency and efficiency is very high

### 1.8.2. Disadvantages of Herbal Drugs

1. Not able to cure rapid sickness and accidents
2. Risk with self-dosing
3. Complexity in standardizations

### 1.8.3. Importance of plants as a source of new drugs[25]

The development of traditional medicinal systems incorporating plants as means of therapy can be traced back to the Middle Paleolithic age some 60,000 years ago as found from fossil Studies. In recent times, developed countries are turning to the use of traditional medicinal systems that involve the use of herbal drugs and remedies and according to the World Health Organization (WHO), almost 65% of the world's population has incorporated the value of plants as a methodology of medicinal agents into their primary modality of health care. It is often noted that 25% of all drugs prescribed today come from plants.

### 1.8.4. Plants used for the treatment of diabetes[26-32]

**Table:3 list of available plants used for the therapy of diabetes mellitus**

| S. NO | BOTANICAL NAME | FAMILY | PARTS USED | MAIN ACTIVE CONSTITUENTS |
|---|---|---|---|---|
| 1. | Allium sativum | Alliaceae | Bulbs | Allyl propyl disulphide, allicin |
| 2. | Annona squamosa | Annonaceae | Fruits | Liriodenine, moupinamide |
| 3. | Areca catechu | Arecaceae | Seed | Arecaine and arecoline |
| 4. | Artemisia pallens | Asteraceae | Leaves and flowers | Germacranolide |
| 5. | Azadirachta indica | Meliaceae | Leaves, flowers & Seed | Azadirachtin and nimbin |
| 6. | Bauhinia forficate | Leguminosae | Leaf | Astragalin, kaempferitrin |
| 7. | Beta vulgaris | Amaranthaceae | Root | Phenolics, betacyanins |
| 8. | Boerhavia diffusa | Nyctaginaceae | Whole plant | Punarnavine and ursolic acid |
| 9. | Camellia sinensis | Theaceae | Leaves | caffeine and catechins |
| 10. | Capparis deciduas | Capparidaceae | Fruit | Spermidine Isocodonocarpine |
| 11. | Cinnamomum Zeylanicum | Lauraceae | Bark | Cinnamaldehyde, eugenol |
| 12. | Combretum Micranthum | Combretaceae | Leaves | Polyphenols |
| 13. | Elephantopus scaber | Asteraceae | Whole plant | Terpenoid and 2,6,23 - Trienolide |
| 14. | Ficus bengalensis Linn | Moraceae | Bark | Leucodelphinidin and Leucopelargonin |

| | | | | |
|---|---|---|---|---|
| 15. | Gymnema sylvestre | Asclepiadaceae | Leaf | Dihydroxy gymnemic triacetate |
| 16. | Gynandropsis Gynandra | Capparidaceae | Root | N,N-diethyltoluamide |
| 17. | Lantana camara | Verbenaceae | Leaves | Lantanoside, lantanone |
| 18. | Liriope spicata | Liliaceae | Root | Beta-sitosterol, stigmasterol |
| 19. | Momordica charantia | Cucurbitaceae | Leaves | Charantin, sterol |
| 20. | Ocimum sanctum | Labiatae | whole plant | Eugenol |
| 21. | Panax quinquefolius | Araliaceae | Root | Ginsenosides, protopanaxadiol |
| 22. | Parinari excels | Chrysobalanaceae | Bark | Myricetin, quercertin |
| 23. | Phyllanthus amarus | Phyllanthaceae | whole plant | Phyllanthin |
| 24. | Prunus amygdalus | Rosaceae | Seeds | Amygdalin |
| 25. | Pterocarpus marsupium | Leguminosae | Whole plant | Kenotannic acid, pyrocatechin |
| 26. | Punica granatum | Lythraceae | Fruit | Punicalagin, punicalin |
| 27. | Ricinus communis | Euphorbiaceae | Root | Ricinolic acid |
| 28. | Salacia oblonga wall | Celastraceae | Root bark | Salacinol |
| 29. | Sarcopoterium Spinosum | Rosaceae | Root | Catechin and epicatechin |
| 30. | Smallanthus Sonchifolius | Asteraceae | Leaves | Sonchifolin, uvedalin, enhydrin, fluctuanin |
| 31. | Swertia punicea | Gentianaceae | Whole plant | Methyl swertianin and Bellidifolin |

| | | | | |
|---|---|---|---|---|
| 32. | Tinospora cordifolia | Menispermaceae | Root | Tinosporone, tinosporic acid |
| 33. | Trigonella foenum Graecum | Fabaceae | Leaves and seeds | 4-hydroxy isoleucine |
| 34. | Vernonia anthelmintica | Asteraceae | Seed | Epoxy acid or vernolic acid |
| 35. | Withania somnifera | Solanaceae | Whole plant | Somniferine,withananine and Cuscohygrine |
| 36. | Aloe barbadenis | Liliaceae | Whole plant | Pseudoprototinosaponin A III and Prototinosaponins AIII |
| 37. | Anemarrhena asphodeloides | Liliaceae | Leaves | Magniferin, Magniferin-7-0-β dglucoside |
| 38. | Bauhinia variegeta | Casealpiniaceae | Aerial parts | Roseoside |
| 39. | Citrullus colocynthis | Cucurbitaceae | Leaves | Beta pyrazol- 1-yalalanine |
| 40. | Ephedra distachya | Ephedraceae | Whole plant | L-ephedrine |
| 41. | Eriobotrya japonica | Rosaceae | Aerial parts | Cinchonain ib |
| 42. | Eugenia jambolona | Mytraceae | Fruit | 4-hydroxy benzoic acid |
| 43. | Accacia Arabica | Fabaceae | Seed, Bark | Polyphenol,Tannins |
| 44. | Cassia auriculata | Fabaceae | Flower | Sterol,terpenoid,flavanoid, Tannins |
| 45. | Glycine max | Fabaceae | Seed | 3-O-methyl-D-chiro-inositol |
| 46. | Tamarindus indica | Fabaceae | Seed, Fruit | Flavonoid, Polysaccharide |

| | | | | |
|---|---|---|---|---|
| 47. | Xanthocercis Zambesiaca | Fabaceae | Leaf | Fagomine, 4-O-beta-Dglucopyranosylfagomine, Castanospermine |
| 48. | Butea monosperma | Fabaceae | Fruit | Butein, Palasonin, Stigmasterol-3 β-D-glucopyranoside |
| 49. | Aegle marmelos | Rutaceae | Leaf, Seed, Fruit | Aegeline 2, Coumarin, Flavonoid, Alkaloid |
| 50. | Citrus reticulate | Rutaceae | Fruit | Essential oil |

### 1.8.5.Marketed Herbal Formulations for the Therapy of Diabetes[33,34]

**Table 4: some herbal formulations for the management of diabetes**

| S.NO | FORMULATION NAME | INGREDIENTS |
|---|---|---|
| 1. | Adcaps | Haldi, Jambuphal, Amla, Mamajov, Neem, Karela, Vijaysar, Tejba, Gulvel Sudha, Guggl, Trivang Nag Suvarnamakshik bhasm, Shilajeet, Ashok and Madhunasni |
| 2. | BZR-34 | Bereris aristata, Ptercarpus marsupium, Gymnema sylvestre, Rubia cordifolia, Trigonella feounum, Berberis aristata, and Tinopora cordifolia |
| 3. | Asanand | Arjuna, Ganasar, Karanja, Kanth, Lodhra, Palash and Shirish |
| 4. | Diasulin | Cassia auriculata, Coccinia indica, Momordica charantia, Syzygium cumini, Emblica officinalis, Trigonella foenum graecum, Curcuma longa, Gymnema sylvestre, Tinospora cordifolia, and Scoparia dulcis |

| | | |
|---|---|---|
| 5. | Diabecure | Berberis vulgaris, Erytherea centaurium, Juglans regia, millefolium and taraxacum |
| 6. | Panvli | Amla, Gudmar, Gul vel, Haldi, Kanth, Karvas, Panvelley and Yashti, |

**1.9. PHYTOSOMES**

From the last century, there are valuable criteria has been kept it up and centred on the event of Novel drug delivery system (NDDS) for herbal medicines. Researchers have the reputable the potential edges of novel drug delivery in providing immense enhancements to deliver the drug and drug targeting. Improving delivery technique, minimize toxicity, improve efficaciousness offers an excellent potential edges to patients and expose new markets for Pharmaceutical and drug corporations. Preparations of plants or parts of them were widely used in popular medicine since ancient times and till today the use of phytomedicines is widespread in most of the world's population. The term 'Phyto' means plant while 'Some' means cell-like. Phytosome is vesicular drug delivery system in which phytoconstituents of herb extract surround and bound by lipid. The Phytosomes process produces a little cell because of that the valuable components of the herbal extract are protected from destruction by digestive secretions and gut bacteria. Phytosomes are better able to transition from a hydrophilic environment into the lipid-friendly environment of the enterocyte cell membrane and from there into the cell finally reaching the blood. Phytosome is a newly introduced patented technology developed to incorporate the standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, which improves their absorption and bioavailability. Phytosomes are more bioavailable as compared to herbal extract owing to their enhanced capacity to cross the lipid rich biomembranes and finally reaching the blood. The phytosome process has been applied to many popular herbal extracts including Ginkgo biloba, grape seed, hawthorn, olive fruits and leaves, milk thistle, green tea, ginseng, kushenin, marsupsin and curcumin. The flavonoid and terpenoid components of these herbal extracts are able to directly bind to phosphatidylcholine. Increased bioavailability of the phytosomes over the simpler,

non-complexed plant extract has been demonstrated by pharmacokinetics and activity studies, conducted in animals as well as human beings[35].

### 1.9.1. Advantages of Phytosomes

- They permeate the non-lipophilic botanical extract to allow better absorption from the intestinal lumen, which is otherwise not possible.
- The formulation of phytosome is safe and the components have all been approved for pharmaceutical and cosmetic use.
- They have been used to deliver liver protecting flavonoids because they can be made easily bioavailable by phytosomes. In addition to this, Phosphatidylcholine is also hepatoprotective and so provides a synergistic effect for liver protection.
- There is no problem with drug entrapment during formulation preparation. Also, the entrapment efficiency is high and moreover predetermined; because the drug itself forms vesicles after conjugation with lipid[36].
- Phosphatidylcholine used in formulating phytosome process besides acting as a carrier also nourishes the skin as it is an essential part of a cell membrane.
- Phytosomes are also superior to liposomes in skin care products.
- Phytosomes proves to be of significantly greater clinical benefit.
- Their low solubility in aqueous media allows the formation of stable emulsions or creams[37].
- Facilitates the liver targeting by increasing the solubility in bile salt.
- Phytosomes are better able to transition from a hydrophilic environment into the lipid-friendly environment of the enterocyte cell membrane and from there into the cell, and thus can be used for systemic targeting.
- Phytosome are widely used in cosmetics due to their more skin penetration and have a high lipid profile[38].

### 1.9.2. Properties of Phytosomes

Following are some of the important properties of phytosomes:

#### 1.9.2.a. Physico-chemical properties

a) Phytosome are prepared by reaction of stoichiometric amount of phospholipid with the standardized plant extracts as substrate. The spectroscopic data reveals that the phospholipid substrate interaction is due to the formation of hydrogen bond

between the polar head (i.e. phosphate and ammonium group) and the polar functionalities of the substrate.

b) The size of Phytosome varies from 50 nm to a few hundred µm.

c) Phytosome when treated with water assumes a micellar shape resembling liposome and photon correlation spectroscopy (PCS) reveals this liposomal structures acquired by phytosome.

d) The H1NMR and C13 NMR data deduced that the fatty chain gives unchanged signals both in free phospholipid and in the complex, which indicates that long aliphatic chains are wrapped around the active principle producing lipophilic envelope.

e) The complexes are often freely soluble in aprotic solvents, moderately soluble in fats, insoluble in water and relatively unstable in alcohol. But the phytosomes of certain lipophilic phytoconstituents like curcumin has shown increase in water solubility upon complexation with phospholipid.

**1.9.2.b. Biological properties**

Phytosome are novel complexes which are better absorbed and utilized; hence they produce more bioavailability and better result than the conventional herbal extract or non-complex extracts, which has been demonstrated by pharmacokinetic studies or by pharmacodynamic tests in experimental animals and in human subjects[39].

### 1.9.3. Bioavailability of Phytosomes

It is evident from many research studies that phytosomes have an improved absorption and bioavailability when compared to the conventional means. Most of the research studies are focused on Silybum marianum (milk thistle), the fruit of which contains a water-soluble phytoconstituent (flavonoids) which is known to have a hepatoprotective effect. But these flavonoids are poorly absorbed. The chief and most potent constituent of milk thistle is Silybin.

A brief summary of some of the research studies is given as:

- According to Crema et al., 1990, when single oral doses of Silybin directly bound to phosphatidylcholine (Silybin phytosome) are fed, its absorption was approximately seven times more than the absorption from regular milk thistle extract containing 70-80% silymarin content).

- A research study was conducted by (Yanyu etal., 2006) in which he prepared silymarin phytosome and has shown its pharmacokinetics in rats. The phytosome was administrated to rats orally. The results showed that the bioavailability and biological effects of Silybin was increased remarkably.
- some of the studies have reported the better results produced by consuming ginkgo phytosome than the conventional gingko extract. A bioavailability study was conducted on healthy human volunteers in which it was found that the levels of flavonoids and terpenes (GBE constituents) peaked after 3 hours and persisted longer last for 5 hours. One study shows that some patients suffering from Reynaud's disease and intermittent circulation were fed with ginkgo phytosome which was shown to produce a 30-60% greater improvement compared to regular standardized GBE (Ginkgo biloba extract)[32].

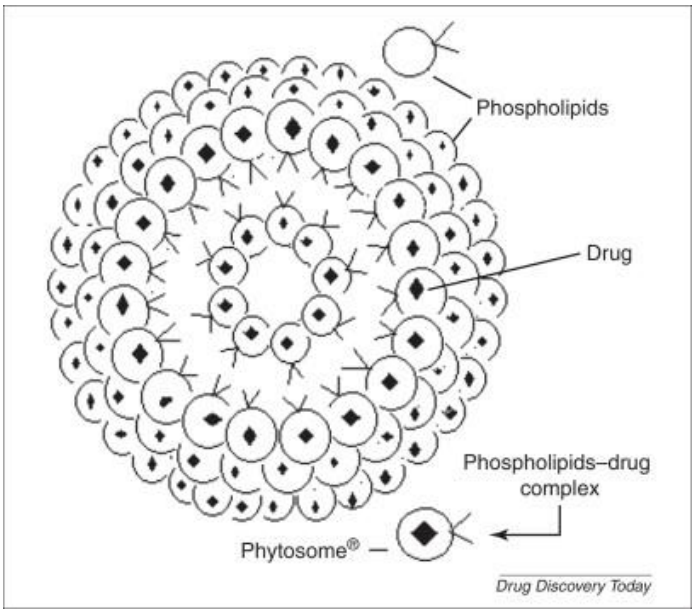


**Figure 5 Phytosome**

### 1.9.4. Applications of Phytosomes[39-44]

To examine the various advantages of phytosomes, especially their ability to enhance the bioavailability of polar phytoconstituents, various therapeutic applications of phytosomes have been explored. The details of the type of phytosomes, active constituents, the daily dose and specific indications are given in (Table 1).

**Table 5: Therapeutic applications of different Phytosomes with their dose**

| S. No. | Phytosomes | Phytoconstituent complexed with PC | Daily dose | Indications |
|---|---|---|---|---|
| 1 | Leucoselect® phytosome | Procyanidolic oligomers (PCOs) from grape seeds | 50-100 mg | Systemic antioxidant, specific. Best choice for most people under age of fifty. Also specific for the eyes, lungs, diabetes, varicose veins, and protection against heart disease. |
| 2 | Greenselect® phytosome | Epigallocatechin 3-O-gallate from camelia sinensis (Green tea) | 50-100 mg | Systemic antioxidant. Best choice for protection against cancer and damage to cholesterol |
| 3 | Ginkgoselect® phytosome | 24% ginkgo flavono glycosides from Ginkgo biloba | 120 mg | Best choice for most people over the age of 50. Protects brain and vascular lining [34] |
| 4 | Silybin phytosome | Silybin from silymarin (milk thistle) | 120 mg | Best choice if the liver or skin needs additional antioxidant protection |
| 5 | Siliphos TM milk thistle phytosome | Silybin from silymarin | 150 mg | Good choice for liver or skin support[35] |
| 6 | Hawthorn phytosome | Flavonoids | 100 mg | Best choice in heart disease |
| 7 | Panax ginseng phytosome | 37.5% ginsenosides from roots of Panax ginseng | 150mg | As a Food Product |

| | | | | |
|---|---|---|---|---|
| 8 | Glycyrrhiza phytosome | 18-beta glycyrrhetinic acid | 50-100mg | Anti-inflammatory Activity[36] |
| 9 | Mirtoselect® Phytosome | Anthocyanosides froman extract of Bilberry | 150mg | These improve capillary tone, reduce abnormal blood vessel permeability & are potent antioxidants. They hold great potential for the management of retinal blood vessel problems and venous insufficiency. |
| 10 | Sabalselect® Phytosome | An extract of sawpalmet toberries through supercriticalCO2 (carbondioxide) extraction | 100mg | It delivers fatty acids, alcohols and sterols that benefit prostate health. Also beneficial for non-cancerous prostate enlargement |
| 11 | PolinaceaTM Phytosome | Echinacosides and a unique high-molecular weight Polysaccharide from Echinacea Angustifolia | 120mg | It enhances immune function in response to a toxic challenge |
| 12 | OleaselectTM Phytosome | Polyphenols from olive oil | 100mg | As potent antioxidants, inhibit harmful oxidation of LDL cholesterol, and also have anti-inflammatory activity. |

# CHAPTER – II

# PLANT PROFILE

## CHAPTER-II

## PLANT PROFILE

**Figure 6: Trigonella foenum graceum**

### 2.1. TAXONOMY OF TRIGONELLA FOENUM GRACEUM[44]

The botanical classification of *Trigonella foenum graceum* is as following,

Kingdom: Plantae

Super division: Angiosperms

Division: Eudicots

Class: Rosids

Order: Fabales

Family: Fabaceae

Subfamily: Faboideae

Tribe: Trifolieae

Genus: Trigonella

Species: Foenum

## 2.2. VERNACULAR NAMES OF TRIGONELLA FOENUM GRACEUM[45]

| | |
|---|---|
| Botanical Name | Trigonella Foenum-graecum |
| English Name | Fenugreek, Sickle Fruit fenugreek, Greek hay |
| Hindi Name | Methi |
| Sanskrit Name | Methika |
| Tamil Name | Vendhayam |
| Tegulu Name | Menthulu |

## 2.3. ETYMOLOGY:

Fenugreek is derived from foenum-graceum which in turn gives the scientific name of this plant. Foenum- graceum means “green hay”. It was named line this because of the strongest smell of this plant, reminiscent of the hay, as extensively cultivated in the mediterranean region.

## 2.4. ORIGIN:

Western Mediterranean native plant and spread throughout the Mediterranean region since ancient times.

## 2.5. HABITAT:

Road sides, uncultivated land, dry grassland, waste land and harvesting lands (grain fields).

## 2.6. GEOGRAPHICAL DISTRIBUTION:

Middle east, Persia, India, China, Central and Southern Europe, Mediterranean region ,North Africa, United states.

## 2.7. MORPHOLOGY[46]:

Fenugreek(Trigonella foenum graceum) is an annual herbaceous plant of the family Leguminosae family ,measuring between 20 and 50 cm upto 100cm high.

**2.7.1. Stem:** Erect,branched,grooved and with little pubescence.

**2.7.2. Leaves:** The leaves are compound, petiolated, consists of three oblong gleaflets, attenuated towards the base and with toothed margins. Size 20-50 x 8 -17 mm.

**2.7.3. Flowers:** The flowers are white or yellow ,grow from the laef axils and are arranged singly or in little groups of two. Corolla 12-18 mm. It flowers in April and May.

**2.7.4. Fruits:** The fruits are pods or legumes narrow and oblong, resembling a sickle.Pod 80-150 x 2-4 mm with elongated beak at the end. The fruits ripen in summer and harvested in autumn.

**2.7.5. Seeds:** Inside of them , there are between 10-20 tiny seeds. Size 3mm. The seeds are approximately yellow, quandragular and with a groove in the central part.

The whole plant gives off an unpleasant odour, reminiscent of hay.

**2.8. USED PARTS:**

**2.8.1. Seeds:** Smal grains are used as a condiment or spice , and in herbal medicines for its medicinal properties. These seeds have an unpleasant taste , So that they are used in small quantities or mixed with other aromatics ,such as curry mix.

**2.8.2. Leaves:** The leaves of this plant are edible. In some oriental restaurants ,they are consumed in the traditional alu methi,made with steamed fenugreek leaves. This delicacy ,little known in the west, is a vegetable particularly rich in choline and betacarotene.

**2.8.3. Green pasture:** Fenugreek is also a fodder plant. Like other pasture legumes , fenugreek increases the production of milk and meat of the cattle. It is a major source of digestable protein of good quality.

**2.9. CHEMICAL CONSTITUENTS[46]:**

- Carbohydrates: Fructose,sucrose,xylose,arabinose,raffinose,stachyose.

- Fibre: It has a high percentage of soluble fibre such as mucilage and galactomannans. This type of fibre ,which accounts for 25-45% of whole grain, provides emollient effect,protects the digestive mucosa and it is a natural intestinal regulator.
- Protein: Fenugreek is high in easily assimilated protein and rich in phosphorus, so it has been indicated as plant tonic .It contains mucins, which are a type of protein with gelling power.
- Amino acids: Cysteine, proline, tryptophan, Phenylalanine and lysine. It also contains 4-hydroxyisoleucine acid with cholesterol lowering properties.
- Fat: It contains lecithin . Among its fatty acid , predominates linoleic acid.
- Minerals: Calcium, phosphorus, Potassium, selenium, Chromium, copper, sodium.
- It contains Trigonelline (Nicotinic acid derivative) and trigonellosides.This substance has hypoglycemic action.
- Aromas: The aroma of the fenugreek is also due to a galactone called sotolone.
- Alkaloids: Carpaine, gentianine.

## 2.10.TRADITIONAL USES[47]:

- Fenugreek is an esteeemed medicine in North Africa, the Middle East, and India, being used for a wide variety of conditions. The nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia.
- Helpful in lowering fever, it is compared to quinine by some authorities.
- The seeds' soothing effect makes them of value in treating gastritis and gastric ulcers.
- They are used to induce childbirth and to increase breast-milk production.
- They are also used to lower blood sugar and blood cholesterol levels.
- Externally, the seeds are applied as a paste to treat abscesses, boils, ulcers, and burns, or used as a douche for excessive vaginal discharge.
- The seeds freshen bad breath and help restore a dulled sense of taste.
- The oil in the seeds is used as a skin softener and emollient.
- In China, the seeds are used as a pessary to treat cervical cancer.

- In the Middle East and the Balkans, the aerial parts are a folk remedy for abdominal cramps associated with both menstrual pain and diarrhea or gastroenteritis. They are also used to ease labour pains.
- Herbalists in Asia and the Mediterranean often recommend fenugreek to stimulate contractions in delayed or sluggish labour

# CHAPTER – III

# LITERATURE REVIEW

## CHAPTER III
## REVIEW OF LITERATURE

1. **Aili Aierken et *al*., (2017)[48]** reported that Hawthorn is a popular herb in many different traditional medicine systems, including traditional Chinese medicine, where it has long been used for the treatment of hyperglycemia. However, most of its varied biological activities remain unexplored. This study investigated the hypoglycemic effect of hawthorn extracts in type II diabetic (T2DM) rat model. A total of 54 rats were randomly divided into six groups: normal control group; type II diabetic model group (T2DM; these rats were induced by high-fat diet and streptozotocin); high, middle and low concentrations of hawthorn treatment ($HT_H$, $HT_M$ and $HT_L$ T2DM rats were given hawthorn extract at a dose of 50, 100 and 200 mg $kg^{-1}$ body weight, respectively); and positive control group (orlistat 40 mg $kg^{-1}$body weight).

2. **Kotha et al., (2017)[49]** investigated that the anti-diabetic activity of leaves of *Anisomeles malabarica* following bioactivity guided fractionation. The different solvent (hexane, ethyl acetate, methanol and water) extracts of *A. malabarica* leaves were used in acute treatment studies to evaluate and identify the active fraction. The ethyl acetate extract was subjected to further fractionation using silica gel column chromatography and the compounds were identified by LC-SRM/MS and GC-MS. Additional chronic treatment studies were carried out using this active fraction (AMAF) for 30 days in experimental diabetic rats. Fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), plasma insulin levels and glucose tolerance were measured along with insulin resistance/sensitivity indicators (HOMA-IR, HOMA-β and QUICKI) to assess the beneficial effects of *A. malabarica* in the management of diabetes mellitus. Among the different solvent extracts tested, ethyl acetate extract showed maximum (66%) anti-hyperglycemic activity. The hexane and ethyl acetate (1: 1) fraction that has maximum anti-diabetic activity was identified as active fraction of *A. malabarica* (AMAF). The FBG, HbA1c, plasma insulin levels and insulin sensitivity/resistance indicators such as glucose tolerance, HOMA-IR, HOMA-β and QUICKI were significantly improved to near normal in diabetic rats treated with AMAF. Further, we identified key flavonoids and fatty acids as the anti-diabetic active principles from the AMAF of *A. malabarica*leaves

3. **Lavakumar *et al.,* (2016)[50]** reported that present investigation was considered in arraying of anti-diabetic and anti-oxidant activity from dietary flavanoid loaded fraction of *Acanthophora spicifera* (Family: *Rhodomelaceae*) on streptozotocin (STZ) induced oxidative stress rats. The testings were acted upon male rats, which were alienated into five groups: control group, diabetic group (single dose of 65mg/kg, STZ i.p.) diabetic with insulin (6IU), and diabetic with flavonoid rich fraction groups (FRF) at 50 and 100mg/kg body weight, given orally for 21 days. The anti-oxidant consequences of FRF on STZ-induced diabetic rats were determined by the estimations of the oxidative stress marker like malnonyldialdehyde and antioxidant enzymes such as superoxide dismutase, catalase and glutathione in tissue homogenates of heart, liver and kidney. FRF treatment of diabetic rats significantly ($P<0.05$) diminishes the blood glucose altitudes to normal in contrast with diabetic rats. However, FRF administration, significantly decreased the malonyldialdehyde (MDA) and increased the activities of superoxide dismutase (SOD), catalase(CAT) and glutathione levels (GSH) in diabetic rats.

4. **Ramadan et al., (2017)[51]** reported that Diabetes is a major public health concern. In spite of continuous new drug development to treat diabetes, herbal remedies remain a potential adjunct therapy to maintain better glycemic control while also imparting few side-effects. *Portulaca oleracea* has been traditionally used to manage several diseases due to the anti-oxidant and anti-atherogenic effects it imparts. To better understand the mechanisms associated with potential protective effect of *P. oleracea* extract against diabetes, alloxan-induced diabetic rats were used in this study. Forty Wistar rats (male, 7–8-wk-old, 140–160 g) were divided into four groups ($n = 10$/group): Group I (control), Group II (*P. oleracea*-treated; gavaged with *P. oleracea* extract daily [at 250 mg/kg] for 4 weeks), Group III (diabetic control; daily IP injection of alloxan [at 75 mg/kg] for 5 days) and Group IV (*P. oleracea*-pre-treated diabetic; gavaged with *P. oleracea* extract daily [at 250 mg/kg] for 4 weeks and then daily IP injection of alloxan [at 75 mg/kg] for 5 days). Body weight, food consumption, blood (serum) levels of glucose, C peptide, Hb A1C, insulin, tumor necrosis factor (TNF)-α and interleukin (IL)-6 were determined for all group

5. **Dhase *et al.,* (2015)[52]** have reported Preparation and Evaluation of Phytosomes containing methanolic extract of Leaves of *Aegle Marmelos (Bael)*. Lipid compatible

molecular complex of extract is called as phytosomes. Phytosome technology is applied to water soluble phytoconstituents tannins, phenols, terpenoids,etc. Phytosomes was prepared by solvent evaporation method and further evaluated it for particle size, digital microscopy, SEM, TEM, FTIR, DSC, XRD analysis. From above studies they have concluded that phytosomes has better physical characteristics as compared to that of methanolic extract of leaves of *A.marmelos*. Phytosomes has nearly same anti-oxidant, anti proliferative and anticancer activity as that of methanolic extract of leaves of *A.marmelos*.

6. **Pedro H. Miranda-Osorio et al., (2016)**[53] reported the effect of C. papaya L. leaf extract (CPLE)on pancreatic islets in streptozotocin (STZ)-induced diabetic rats, as well as on cultured normalpancreatic cells with STZ in the medium. CPLE (3–125 mg/Kg) was administered orally for 20 days,while a group of diabetic rats received 5 IU/Kg/day of insulin. At the end of the treatment the rats were sacrificed. Blood was obtained to assess glucose and insulin levels. The pancreas was dissected to evaluate _ cells by immunohistochemistry. In addition, normal pancreatic cells were cultured in a medium that included CPLE (3–12 mg). One half of the cultured cells received simultaneously CPLE and STZ (6 mg), while the other half received CPLE and five days later the STZ. After three days of incubation, insulin was assayed in the incubation medium. The CPLE administered to diabetic rats improved the fasting glycemia and preserved the number and structure of pancreatic islets. However,when CPLE was added to pancreatic cells in culture along with STZ, the insulin concentration was higher in comparison with the cells that only received STZ.

7. **Moses soloman agawaya et al., (2016)**[54] evaluated the effect of aqueous root bark extract of Zanthoxylum chalybeum on oral glucose tolerance and pancreas histopathology in alloxanized rats.Diabetes was induced in rats by administration of alloxan monohydrate. Root extract of Z. chalybeum was administered to rats at 200 and 400 mg/kg BW daily for 28 days. Blood glucose was measured by glucometer and pancreatic histopathology evaluated microscopically. Initial increase was observed in blood glucose of the rats after oral administration of glucose from time zero. Two hours after treatment with Z. chalybeum, a significant reduction in blood glucose was observed within treatment groups ($p<0.05$) compared to 0.5 hr and 1 hr. There was no significant difference between treatment group receiving 400mg/Kg

BW extract and the normal groups (p=0.27), implying that the former group recovered and were able to regulate their blood sugar, possibly via uptake of glucose into cells. The reversal in pancreatic histopathology further supports the protective effect of Z. chalybeum extract towards diabetic damage. Conclusion. Extract of Z. chalybeum is effective in controlling blood glucose in diabetes and protecting pancreatic tissues from diabetic damage.

8. **Maisa MA Al-Qudah et al., (2016)[55]** reported the biochemical and histological changes in the pancreas of rats exposed to aqueous extract of ginger. Twenty adult female albino rats were divided into four groups of five each: Group I, the control group. Group II: Untreated diabetic rats (injected with 65mg/kg Body Weight (BW) of alloxan intraperitoneally); received distilled water. Group III: Diabetic rats; received 500 mg/kg BW/day of the ginger extract. Group IV: Diabetic rats; received 1000 mg/kg BW/day of the ginger extract. The treatment continued for 21 days, then rats were anesthetized, and their pancreases were extirpated to be processed for light microscopic examinations. The alloxan induced rats exhibited hyperglycemia accompanied with increases in glucose, Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C) and Triglycerides (TG) levels. On the other hand, there were reductions in insulin and High Density Lipoprotein Cholesterol (HDL-C) levels. Ginger was effective in lowering serum glucose and returned the other earlier mentioned blood assays levels in the ginger treated diabetic rats to almost normal value..

9. **Manjeshwar Shrinath Baliga et al., (2017)[56]** reported Diabetes mellitus, a metabolic disorder of the endocrine system is one of the World's oldest diseases known to man. Since time immemorial plants have been used as anti-diabetic agents in the various traditional systems of medicine. Trigonella foenum-graecum commonly known as fenugreek in English and methi in various Indian languages is one such plant and has been an integral part in the treatment of diabetes in the Indian traditional system of medicine the Ayurveda. The seeds and leaves have been documented to be useful in reducing hyperglycemia and its complications. This review collates the traditional uses and validated anti-diabetic effects of the fenugreek leaves and on the mechanisms contributing to the therapeutic effects.

10. **Singh *et al.,* (2015)**[57] reported that *Lawsonia inermis L.* (Lawsone) has low bioavailability because it is less soluble in water and it is rapidly eliminated from body. The aim of this study was to prepare the phytosome of lawsone and evaluate it. Different phytosome complexes of lawsone containing molar ratio of 1:1, 1:2, 2:1 and 2:2 of lawsone and soya lecithin were prepared by the anti-solvent precipitation technique. The phytosome was characterized by SEM, DSC and FT-IR. Antifungal activity of phytosome of lawsone was evaluated on Candida albicans (NCIM 3471) fungi by using ketoconazole as standard drug. SEM and DSC data showed that phytosome complex of lawsone has irregular size vesicles consisting of soya lecithin and lawsone was found to be intercalated in the lipid layer. Antifungal activity of phytosome complex (1:1) showed the biggest zone of inhibition as compared to phytosome complex (1:2), plant drug and standard drug ketoconazole after 3 days. The anti-inflammatory activity of gel of phytosome of lawsone showed significant anti-inflammatory activity as compared to plant drug gel at 4 h ($P < 0.001$).

11. **Hamidpour et al., (2016)**[58] reported that this minireview article analyzes the medicinal and remedial properties of Trigonella foenum graceful L. (fenugreek) as an alternative remedy for diabetes mellitus. To understand fenugreek and the components of fenugreek seed extract (dysgenic, ethanol extract, hydro alcoholic extract, aqueous extract and methanol extract, IND01) as a remedial agent for diabetes mellitus in various in vitro and in vivo studies.

12. **Kailash et al.,(2017)**[59] carried out an anti-diabetic effect to compare anti-diabetic effect between the different aqueous extracts of green leafy plants locally available in North Karnataka region of India on alloxan induced diabetic male albino rats.: The preparation of aqueous extracts, preliminary phytochemical analysis and toxicity screening test of 3 aqueous extracts was done by using standard protocol. To study anti-diabetic activity experimental rats were divided into five groups viz. Group I (Control), Group II (Diabetic, Alloxan monohydrate, 15mg/100g bwt, i.p.), Group III (Diabetic with Trigonella foenum graecum), Group IV (Diabetic with Hibiscus cannabinus Linn) and Group V (Diabetic with Cicer arietinum). All above extracts were supplemented with same dose i.e. 12.5mg/100g bwt, orally. The blood glucose levels were evaluated in all the above experimental groups after acute (OGTT) and sub chronic (2 weeks) supplementation. Results: Our results depicts

statistically significant decreased blood glucose level in Group III rats after both acute and sub chronic supplementation whereas in Group IV rats only after sub chronic supplementation when compared with Group II rats. But, Group V rats not showed any significant change in both acute as well as sub chronic exposure when compared with Group II rats. Trigonella foenum graecum and Hibiscus cannabinus Linn. leaves may be used as a dietary supplement in diabetic patients.

13. **Sapneh Sharma et al., (2015)[60]** reported that this study was undertaken to evaluate the antihyperglycemic and antioxidative potential of seed powder of *Trigonella foenum-graecum* L in alloxan (55 mg/kg) induced diabetic rats. The results obtained showed that extensive oxidative stress is generated in tissues of diabetic rats as evidenced by increased production of hydrogen peroxide, increased accumulation of malondialdehyde (MDA) and 4-hydroxynonanal (4HNE) and decreased activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) in tissues of diabetic rats. It was observed that the transcription of genes of SOD, GPx, and CAT was also significantly decreased when compared with control. Treatment of *Trigonella* for 15 days to diabetic rats showed hypoglycemic effect and improved the altered levels of $H_2O_2$, MDA, and 4HNE, the activities of SOD, GPx, and CAT as well as transcription of these genes in the liver and the brain of diabetic rats.

14 **Piero NM, et al., (2015)[61]** reported this study was to determine in vivo hypoglycemic activity and safety of aqueous leaf extracts of C. pareira in white male albino rats. The extracts were screened for their hypoglycemic activity in alloxan induced diabetic rats using the oral and intraperitoneal routes. The safety of these extracts was studied in rats orally or intraperitoneally administered with 1 g/kg body weight daily for 28 days by recording the changes in body and organ weight, hematological and biochemical parameters and histology. Mineral composition of the extracts were estimated using total reflection X-ray fluorescence system (TRXF) while the types and quantities of phytochemicals present were assessed using standard procedures. Aqueous extracts orally and intraperitoneally administered at 50 mg/kg, 100 mg/kg and 150 mg/kg body weight demonstrated hypoglycemic activity with the intraperitoneal route being more effective than the oral route. Oral and intraperitoneal dose of 1 g/kg body weight of the leaf extracts significantly

reduced the body weight gain. The same intraperitoneal dose increased the liver and spleen, and decreased the testis weight; and reduced the hemoglobin levels, packed cell volume and increased the platelet count; increased the activity of aspartate aminotransferase, and lactate dehydrogenase, and decreased the activity of alkaline phosphatase, γ-glutamyltransferase, and creatine kinase and histologically slightly injured the liver and spleen and orally increased the activity of alanine aminotransferase, lactate dehydrogenase, and creatine kinase, and decreased the activity of aspartate aminotransferase and γ-glutamyltransferase. The extracts contained phenols, tannins, flavonoids, alkaloids, terpenoids, sterols, and reducing sugars. Potassium, calcium, and iron levels in the extracts were below the recommended daily allowance. In conclusion, the observed hypoglycemic activity and slight toxicity could be associated with the phytonutrients present in this plant extract. This study recommends continued use of its plant as herbal medicine.

15. **Gupta *et al.,* (2010)**[62] studied that Curcumin is a major constituent of rhizomes of *Curcuma longa*. Pharmacokinetic studies of curcumin reveal its poor absorption through intestine. Objective of the present study was to enhance bioavailability of curcumin by its complexation with phosphatidyl choline (PC). Complex of curcumin was prepared with PC and characterized on the basis of solubility, melting point, Differential scanning calorimetry, Thin layer chromatography, and Infrared spectroscopic analysis. Everted intestine sac technique was used to study *ex vivo* drug absorption of curcumin–PC (CU–PC) complex and plain curcumin. Pharmacokinetic studies were performed in rats, and hepatoprotective activity of CU–PC complex was also compared with curcumin and CU–PC physical mixture in isolated rat hepatocytes. Analytical reports along with spectroscopic data revealed the formation of complex. The results of *ex vivo* study show that CU–PC complex has significantly increased absorption compared with curcumin, when given in equimolar doses. Complex showed enhanced bioavailability, improved pharmacokinetics, and increased hepatoprotective activity as compared with curcumin or CU–PC physical mixture.

16. **Ranveer Singh Tomar et al., (2014)**[63] Diabetes mellitus is characterised by rise in blood sugar levels resulting from insulin dysfunction or insulin insufficiency. Aim: The aim of the present investigation is to evaluate antidiabetic activity of

hydroalcoholic extract of Annona squamosa Linn (A. squamosa Linn) in alloxan-induced diabetic rat model. Diabetes is induced by a single-dose intraperitoneal injection (i.p.) of alloxan (120 mg/kg) to albino rats. Treatment with A. squamosa Linn. extract at a dose of 350 mg/kg and 700 mg/kg and Metformin at a dose of 5mg/kg for 28 days, after induction of diabetes by alloxan, caused significant reduction in blood serum glucose and serum lipid profiles like total cholesterol and triglycerides but significant increase in body weight and serum high density lipoproteins (HDL) level in diabetic rats compared to untreated group. Histological study of the pancreas of diabetic rat treated with A. squamosa extract also showed partial regeneration of beta cells. The antidiabetic activity of this extract is found comparable to Metformin. Thus, leaves of A. squamosa Linn. can be used as potential antidiabetic drug

17. **Yingli Jin et al., (2014)**[64] The present study aims to examine the protective effect of fenugreek and the underlying mechanism against the development of diabetic nephropathy (DN) in streptozotocin- (STZ-) induced diabetic rats. A rat model of diabetes was successfully established by direct injection of STZ and then the rats were administered an interventional treatment of fenugreek. Parameters of renal function, including blood glucose, albuminuria, hemoglobin A1c (HbA1c), dimethyl formamide (DMF), blood urine nitrogen (BUN), serum creatinine (Scr), and kidney index (KI), were detected in the three groups (Con, DN, and DF). Oxidative stress was determined by the activity of antioxidase. Extracellular matrix (ECM) accumulation and other morphological alterations were evaluated by means of immunohistochemistry and electron microscope. Quantitive (q)PCR was employed to detect the mRNA expression of transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) and protein expression was determined with western blot analysis. DN rats in the present study demonstrated a significant renal dysfunction, ECM accumulation, pathological alteration, and oxidative stress, while the symptoms were evidently reduced by fenugreek treatment. Furthermore, the upregulation of TGF-β1 and CTGF at a transcriptional and translational level in DN rats was distinctly inhibited by fenugreek. Consequently, fenugreek prevents DN development in a STZ-induced diabetic rat model.

18. **Baynes et al.,(2015)[65]** Diabetes Mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia either immune-mediated (Type 1 diabetes), insulin resistance (Type 2), gestational or others (environment, genetic defects, infections, and certain drugs). According to International Diabetes Federation Report of 2011 an estimated 366 million people had DM, by 2030 this number is estimated to almost around 552 million. There are different approaches to diagnose diabetes among individuals, The 1997 ADA recommendations for diagnosis of DM focus on fasting Plasma Glucose (FPG), while WHO focuses on Oral Glucose Tolerance Test (OGTT). This is importance for regular follow-up of diabetic patients with the health care provider is of great significance in averting any long term complications.

19.**Karthikeyan et al., (2016)[66]** Diabetes mellitus is a group of metabolic disorder, is characterized by absolute by lack of insulin and resulting in hyperglycemia. About 2.8% of global populations are affected by Diabetes mellitus. The search for new drug with new properties to treat the disease is still in progress. The current review have made an attempt to bring together all reported models and advanced techniques. Experimentally diabetes mellitus is generally induced in laboratory animals by several methods that include: chemical, surgical and genetic manipulations. The various *in vitro* techniques includes: *In-vitro* studies on insulin secretion, *In-vitro* studies on glucose uptake, Studies using isolated pancreatic islet cell lines, Assay of Amylase Inhibition and Inhibition of α-Glucosidase Activity. Experimental induction of diabetes mellitus in animal models and *in vitro* techniques are essentials for the advancement of our knowledge, clear understanding of pathogenesis and for finding new therapy. The animal models and *in vitro* techniques are essentials for developing a new drug for the treatment diabetes. More animal models, advanced techniques have to be developed for advances in diabetes research.

20. **Naseer Ali Shah et al., (2014)[67]** Reported that the ethyl acetate fraction (SCEE) of Sida cordata was investigated for scientific validation of its folk use in diabetes. Antidiabetic effect of SCEE was confirmed by antihyperglycemic activity in normal glucose loaded and diabetic glucose loaded animals as well as normal off feed animals. Confirmation of antidiabetic activity and toxicity ameliorative role of S.

cordata was investigated in a chronic multiple dose treatment study of fifteen days. A single dose of alloxan (120 mg/kg) produced a decrease in insulin level, hyperglycemia, elevated total lipids, triglycerides, and cholesterol and decreased the high-density lipoproteins. Concurrent with these changes, there was an increase in the concentration of lipid peroxidation (TBARS), $H_2O_2$, and nitrite in pancreas, liver, and testis. This oxidative stress was related to a decrease in glutathione content (GSH) and antioxidant enzymes. Administration of SCEE for 15 days after diabetes induction ameliorated hyperglycemia, restored lipid profile, blunted the increase in TBARS, $H_2O_2$, and nitrite content, and stimulated the GSH production in the organs of alloxan-treated rats. He suggested that SCEE could be used as antidiabetic component in case of diabetes mellitus. This may be related to its antioxidative properties.

21**. Ndidi et al., (2013)**[68] reported the tissue-protective potential of Persea americana necessitated a look into the histopathological effects of the plant extract on the pancreas, liver, and kidneys. This study was conceived and designed based on the gaps in the research that has been performed and what is known about the plant. The hypoglycaemic and tissue-protective effects of hot aqueous Persea americana (avocado pear) seed extracts on alloxan-induced albino rats were investigated. Persea americana seeds were extracted using hot water, and different concentrations of the extract were prepared. The effects of different concentrations (20, 30, 40 g/L) of the hot aqueous P. americana seed extract on alloxan-induced Wistar albino rats were compared with those of a reference drug, Metformin. The glucose level of the rats was measured daily, and the weight of the animal was monitored on a weekly basis for 21 days. The oral glucose tolerance test (OGTT) was performed at 0, 30, 60, 90 and 120 minutes, and the histopathologies of the liver, kidneys, and pancreas were investigated. Phytochemical analysis of P. americana seed extracts indicated the presence of glycosides, tannins, saponins, carbohydrates, flavonoids, and alkaloids. The results showed that the extract possessed a significant hypoglycaemic ($P < 0.05$) effect and reversed the histopathological damage that occurred in alloxan-induced diabetic rats, comparable to the effects Metformin. The seeds of P. americana also had anti-diabetic and protective effects on some rat tissues such as the pancreas, kidneys, and liver.

22. **Lachin et al., (2012)[69]** reported that Diabetes mellitus (DM) is a metabolic disorder in the endocrine system resulting from a defect in insulin secretion, insulin action or both of them. Adverse side effects of chemical drugs for treatment of diabetes persuaded the using of medical plants. Cherry as a traditionally used plant for treatment of diabetes, is packed with powerful plant pigments called anthocyanins. They give cherries their dark red color and are one of the richest antioxidant sources which lower the blood sugar and bear other beneficial health effects. The purpose of this study is to evaluate the effect of ethanolic extract of cherry fruit on alloxan induced diabetic rats. In this study 36 Male Wistar rats, body weight of 150-200gr were divided into 6 groups. Diabetes was induced by intra peritoneal injection of 120 mg/kg Alloxan. The duration of the cherries treatment was 30 days in which single dose of extracts (200mg/kg) were oral administered to diabetic rats. Blood glucose levels were estimated with glucometer before treatment, 2h and 1- 4 weeks after administration of extracts. Treatment with extracts of the cherries resulted in a significant reduction in blood glucose and urinary microalbumin and an increase in the creatinine secretion level in urea. Extract of this plant is useful in controlling the blood glucose level. Cherries appear to aid in diabetes control and diminution of the complications of the disease.

23. **Sornalakshmi et al.,(2016)[70]** reported The effect of ethanol extract of whole plant of Hedyotis leschenaultiana on Oral Glucose Tolerance was determined. Metformin (600µg/kg) was used as reference drug for comparison. Ethanol extract of Hedyotis leschenaultiana whole plant was evaluated for Oral Glucose Tolerance Test (OGTT) in normal and alloxan induced diabetic rats. Blood glucose concentration was evaluated at 0, 30, 60, 90 and 120 minutes after treatment in both cases. The extract significantly ($p < 0.001$) reduced blood glucose level in alloxan induced diabetic (hyperglycaemic) rats orally at the dose of 150mg/kg and 300mg/kg body weight of ethanol extract respectively. These results suggest that the ethanol extract of Hedyotis leschenaultiana whole plant will be useful in the treatment of impaired oral glucose tolerance.

# CHAPTER – IV

# AIM AND OBJECTIVE

# CHAPTER IV

# AIM AND OBJECTIVE

## 4.1. AIM

The aim of the study is to Prepare and evaluate the Tigonella foenum graceum nanophytosomes for Anti-Diabetic activity on Wistar rats.

## 4.2. OBJECTIVES

To emphasize the above aim, I have followed certain objectives

- Extraction of leaves of *Trigonella foenum graceum* and its phytochemical investigation.
- Preparation of nanophytosomes of *Trigonella foenum graceum*.
- Performing the *In vitro* and *In vivo* anti-diabetic activity of *Trigonella foenum – graceum* nanophytosomes.

## 4.3. PLAN OF WORK

The detailed plan of work laid into the following levels,

I. Collection and Extraction of *Trigonella foenum -graceum* leaves by using water as a solvent

II. The extracts are concentrated and subjected for preliminary phytochemical investigation.

III. The nano-phytosomes are prepared by using *Trigonella foenum -graceum* leaf extract by thin layer lipid hydration method

IV. The prepared nano-phytosomes are subjected for Characterization studies such as,

   a. Scanning Electron Microscope (SEM)

   b. Fourier Transform – Infra Red Spectroscopy(FT-IR),

   c. Differential Scanning Calorimetry (DSC)

   d. Particle size and Zeta potential(PS & ZP)

   e. Transmission Electron Microscopy (TEM)

   f. X-Ray Diffractometer (XRD)

V. The *Trigonella foenum -graceum* nano-phytosomes and aqueous extract are subjected for *In vitro* Anti-Oxidant activity such as,

   - Nitric Oxide Radical scavenging activity (NO)

   - Ferric reducing Anti-Oxidant Power Assay (FRAP)

   - Total Anti-Oxidant activity (TAC)

VI. The *Trigonella foenum -graceum* nano-phytosomes and aqueous leaf extract are screened for *In vitro* Anti-diabetic activity

VII. The *Trigonella foenum -graceum* nano-phytosomes and aqueous leaf extract are screened for *In vivo* Anti-diabetic activity by Streptozocin induced diabetes rat model.

VIII. Bio-chemical studies involves the withdrawing the blood and estimated for following parameters like,

a. Estimation of Super Oxide Dismutase Activity (SOD)

b. Estimation of Catalase activity (CAT)

c. Estimation of Reduced Glutathione (GST)

d. Thio Barbituric Acid Reactive Substances (TBARS)

IX. Histopathology studies

# CHAPTER – V

# MATERIALS AND METHODS

# CHAPTER V

# MATERIALS AND METHODS

## 5.1. MATERIALS

Cholesterol was obtained from Loba Chem lab Pvt. Ltd., Maharashtra; Egg lecithin is obtained from Lipoid pharma Pvt. Ltd., Germany; Chloroform was obtained from Sisco Research Laboratories Pvt. Ltd., New Mumbai; Streptozocin was obtained from Glenmark Pharmaceuticals Ltd., Goa; Metformin was obtained from Ipca Laboratories Ltd., Mumbai; Formalin was obtained from Thermo Fisher Scientific India Pvt. Ltd., Mumbai;Fenugreek leaves were collected from local market of Rajapalayam. All the chemicals and reagents used in this experiment are of analytical grade.

### 5.2.1. COLLECTION AND PROCESSING OF *TRIGONELLA FOENUM GRACEUM* PLANT MATERIAL

*Trigonella foenum graceum* leaves were collected locally from the Rajapalayam market (Virudhunagar Dist,Tamilnadu). The leaves were separated from the plant and washed with water and chloroform to remove soil particles, spread them and dried in the shade for 7 days.

### 5.2.2. PREPARATION OF LEAF EXTRACT[70]

The shade dried leaves were subjected to size reduction by trituration by using mortar and pestle to make into fine powder. Weigh required quantity of powder and it is dissolved in a requied quantity of boiling water. Then the mixture of powder and water is placed in a shaker incubator for 24 hrs.Then it is filtered by using whatmann filter paper.Then the filtrate was concentrated on water bath at $40^0$c for 2days.Then finally extract was collected and stored at $4^0$c.

### 5.2.3. PRELIMINARY PHYTOCHEMICAL ANALYSIS[71]

The aqueous extract of leaves of*Trigonella foenum graceum* obtained was subjected to qualitative analysis to test the presence of various phytochemical like alkaloids, flavonoids, steroids, phenols, proteins and amino acids, terpenoids,anthraquinones and quinones etc.

**A) PROCEDURE**

**(I) TEST FOR ALKALAOIDS**

**a.) Mayer's test**

A fraction of extract was treated with mayers test reagent(1.36 g of mercuric chloride and 5g of potassium iodide in 100ml water) and observed for the formation of cream coloured precipitate.

**b.) Wagner's test**

A fraction of extract was treated with wagner's test reagent(1.27 g f iodide and 2g of potassium iodide in 100 water) and observed for the formation of reddish brown coloured precipitate.

**c.) Hager's test**

A few ml of extract was treated with hager's test reagent(saturated aqueous solution of picric acid) and observed for the formation of prominent yellow coloured precipitate.

**(II) TEST FOR FLAVANOIDS**

**a.) NaoH test**

A small amount of extract was treated with aqueous NaoH and HCl,observed for the formation of yellow orange colour.

**b.) $H_2SO_4$ test**

A fraction of extract was treated with concentrated $H_2SO_4$ and observed for the formation of orange colour.

**(III) TEST FOR STEROLS**

**a.) Liebermann-Burchard test**

A fraction of extract was treated with chloroform,acetic unhydride and drops of concentrated $H_2SO_4$ was added and observed for the formation of pink or red colour.

**(IV) TEST FOR TERPENOIDS**

**a.) Liebermann test**

A fraction of extract was treated with chloroform,acetic unhydride and drops of concentrated $H_2SO_4$ was added and observed for the formation of greyish colour.

**(V) TEST FOR PROTEINS AND AMINO ACIDS**

**a.) Ninhydrin test(aqueous)**

A fraction of extract was treated with aqueous ninhydrin and observed for the formation of blue colour, indicating presence of amino acids or purple colour indicating the presence of protein.

**b.) Ninhydrin test(acetone)**

Ninhydrin was dissolved in acetone,the extract was treated with ninhydrin and observed for the formation of purple colour.

**c.) Biuret test**

The extract was heated in distiiled water and filtered. The filtrate is treated with 2%copper sulphate solution,to this added 95%ethanol and potassium hydroxide and observed the formation of pink colour.

**(VI) TEST FOR ANTHRAQUINONES**

**a.) Borntrager's test**

About 50mg of powdered extract was heated with 10%ferric chloride solution and 1ml concentrated HCl. The extract was cooled,filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia;pink or deep red colour of aqueous layer indicate the presence of anthraquinones.

**(VII) TEST FOR PHENOLS**

**a.) Ferric chloride test**

A fraction of extract was treated with 5% ferric chloride and observed for the formation of deep black or blue colour.

**b.) Liebermann's test**

The extract was heated with sodium nitrite,added $H_2SO_4$ solution diluted with water and excess of dilute NaoH was added and observed for the formation of deep red or green or blue colour.

**(VIII) TEST FOR QUINONES**

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow coloured precipitate.

**B.THIN LAYER CHROMATOGRAPHY [72]:**

Thin layer chromatography (TLC) technique for separation of active compounds was carried out by the method of Hao et al. (2004). Slurry was prepared by mixing 30gm of silica gel G with 100ml of water. The slurry was poured into glass plate and the slurry was spreaded uniformly on the surface of the glass plate. After setting the glass plates were dried in hot air oven at $110^0$c for 1hr. Baseline was drawn on the TLC plate.Small spot of solution containing the sample is applied to a plate and dried. Small amount of an appropriate solvent (eluent) Methanol : Chloroform (1:9) which shows better separation of compounds, poured in to a TLC chamber to a depth of less than 1 cm. The container is closed with a cover glass or lid and is left

for 10 minutes for saturation. The TLC plate is then placed in the chamber and allowed to run the chromatogram. The solvent moves up the plate by capillary action meets the sample mixture and carries it up the plate (elutes the sample). The dried plate is placed in a chamber containing a few crystals of iodine. The iodine vapor in the chamber oxidizes the substances in the various spots making them visible to the eye. Once the spots are visible they may be outlined with a pencil before the iodine coloration fades.

$R_f$ value = $\frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$

### 5.2.4. PREPARATION OF NANOPHYTOSOMES

**Procedure**

**a)THIN LAYER LIPID HYDRATION METHOD[73]:**

Accurately weighed quantity of egg lecithin and cholesterol were dissolved in 10 ml of chloroform in round bottom flask (RBF) and sonicated for 10 min using bath sonicator. Organic solvent removal is done by Rotary evaporator (45-50°C). After complete removal of solvent thin layer of phospholipids mixture was formed. This film was hydrated with Aqueous extract of *Trigonella foenum graceum* leaves in rotary evaporator (37-40°C for 1 hour). After hydration, mixture of lipid and plant extract was sonicated for 20 minutes in presence of ice bath for heat dissipation. Then prepared phytosomes were filled in amber colored bottle and stored in freezer (2-8 $^0$C) until used.

**b) Preparation of phytosome complexes of *Trigonella foenum graceum*[74]**

The different phytosome complexes of *Trigonella foenum graceum* F1, F2, F3 & F4 containing molar ratio of 1:0.5:1, 2:1:1, 1:0.5:2 and 2:1:2 of Egg lecithin, Cholesterol and Trigonella foenum graceum extract were prepared as mentioned in **table 6**

Composition of phytosome formulation of aqueous extract of *Trigonella foenum graceum l*eaves

**Table 6: formulation of nanophytosomes**

| Phytosomes | Molar ratio (EL:CL:EX) | Chloroform |
|---|---|---|
| F1 | 1:0.5:1 | 10ml |
| F2 | 2:1:1 | 10ml |
| F3 | 1:0.5:2 | 10ml |
| F4 | 2:1:2 | 10ml |

**EL: Egg lecithin CL: Cholesterol EX:Extract**

### 5.2.5. CHARACTERIZATION OF NANOPHYTOSOMES:

#### a)SEM analysis[75]

Scanning electron microscopy has been used to determine particle size distribution and surface morphology of the complexes. Samples were studied using JEOL JSM-6360 Scanning microscope (Japan). Approximately 5 µL of the phytosomal suspension was transformed to a cover slip, which in turn was mounted on a specimen tab. The samples were allowed to dry at room temperature. Then the particle size of the formulation was viewed and photographed using Scanning Electron Microscope (Sigma, Carl Zeiss). The particles were coated with platinum by using vaccum evaporator and thus, the coated samples were viewed and photographed in JEOL JSM-6701F Field Emission SEM. Digital images of phytosome complex of *Trigonella foenum graceum* were taken by random scanning of the stub at 1000, 5000, 10000 and 30000 X magnifications.

#### b) DSC analysis[75]

Thermodynamical techniques are applied for determining the thermal stress of medicinal compounds of the excipients as well as their interactions during the formulation process. The thermal analysis of the *Trigonella foenum graceum*, physical mixture of egg lecithin and cholesterol, physical mixture of egg lecithin and cholesterol and phytosome complex of *Trigonella foenum graceum* were placed in the aluminum crimp cell and heated at 10 $^0$C/min from 0 to 400$^0$C in the atmosphere of nitrogen (TA Instruments, USA, model DSC Q10 V24.4 Build 116). Peak transition onset temperatures were recorded by means of an analyzer. *Trigonella foenum graceum* leaves extract, phospholipon and phytosome were placed in the aluminum

crimp cell and heated at 100C/min from 0 to 400 0C in the atmosphere of nitrogen (TA Instruments, USA, Model DSC Q10 V24.4 Build 116). Peak transition onset temperatures were recorded by means of an analyzer.

**c) FT-IR analysis[78]**

FT-IR spectral data can be taken to determine the structure and chemical stability of pure drug, physical mixture of egg lecithin and cholesterol, physical mixtures and nanophytosomal formulations. The spectroscopic evaluation of the formed complex can be confirmed by FTIR simply by comparing the spectrum of the complex and the individual components and that of the mechanical mixtures. Samples were mixed with dry crystalline KBr in a ratio of 1:100 and pellets were prepared. The mixture was grounded or triturated into fine powder using an agate mortar before compressing into KBr disc. Each KBr disc was scanned at 4 mm/s at a resolution of 2. FTIR can also be considered as a valuable tool in confirming the stability of the phytosomal complex. FT-IR spectra were obtained using a FT-IR spectrometer. Spectral scanning can be done in the range between 4000-400 cm-1.

**d) XRD analysis[77]**

XRD is a unique method in determination of crystallinity of a compound and when properly interpreted, by comparison with drug XRD pattern before formulation., allows the identification of the drug crystalline changes. XRD was done on pure extract, physical mixtures of egg lecithin and cholesterol, physical mixtures and phytosome to see the crystallinity in the substance. Sample was scanned in the angular range of 50 - 800 in a PHILIPS XPert Pro X-Ray Diffractometer. Dried powder sample was kept in sample holder (20 mm × 15mm × 2mm) which was fitted into the instrument and X-ray was passed through the sample.

**e) Particle size and zeta potential[76]**

The particle size and Zeta potential of *Trigonella foenumgraceum* Nano-phytosome were assayed by dynamic light scattering (DLS) using a Malvern Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK). Scattered light was detected at a 173° angle. All measurements were performed in the standard suitable conditions to validate the results of sample dilution and the constant temperature of 25 °C. Furthermore, the apparatus we used systematically and automatically adapts to the

sample, the intensity of the laser as well as the attenuator. Therefore, the reproducibility of the experimental measurement conditions is ensured.

**f)TEM analysis[76]**

Vesicles morphology of phytosome was observed visually with a JEOL JEM 1400 (Japan) Transmission Electron Microscopy (TEM). A total volume of 10 mL sample was dispersed before the sample was analyzed. The mixture was then stirred and a drop of the sample was placed on the specimen. The 400 mesh grid was placed over the specimens and allowed to stand for 1 minute. Residual droplets on the grid were cleaned using a filter paper. A drop of uranyl acetate was dropped over the grid and the rest of the excess solution was removed using a filter paper. The grid was left for 30 minutes and the films were then viewed on a transmission electron microscope and photographed.

## 5.2.6. INVITRO ANTIOXIDANT ACTIVITY OF *TRIGONELLA FOENUM – GRACEUM* NANOPHYTOSOMES:

The following *In vitro* models were carried out to evaluate antioxidant activity

- Nitric oxide radical scavenging activity(NO)
- Ferric Reducing Anti-oxidant Power Assay (FRAP)
- Total Anti-oxidant activity(TAC)

**a) Nitric oxide radical scavenging activity(NO)[79]**

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radical. The Nitric oxide radical scavenging method of *Trigonella foenum graceum* nano-phytosomes and extract was determined method. Nitric oxide (NO) radical were generated from sodium nitroprusside solution at physiological pH Sodium nitroprusside (1ml of 10mM) were mixed with 1ml of *Trigonella foenum graceum* nano-phytosomes and extract of different concentration like 50µg, 100µg, 150µg, 200µg, 250 µg in phosphate buffer (pH 7.4). The mixture

was incubated at 25° C for 150 min. To 1 ml of the incubated solution, 1ml of Griess's reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm. BHA (Jayaprakasha et.al., 2004) used as a positive control. % inhibition of OD is calculated by using the formula,

$$\% \text{ scavenging} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

**b) Ferric reducing antioxidant power (FRAP) assay[80]**

FRAP assay is based on the ability of antioxidants to reduce Fe3+ to Fe2+ in the presence of 2,4,6-tri(2-pyridyl)s-triazine (TPTZ), forming an intense blue Fe2+-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content (Benzie and Strain, 1996). 0.2 ml of *Trigonella foenum graceum* nano-phytosome and extract is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM FeCl3. 6H2O solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. FeSO4 is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mmol FeSO4 equivalents per gram of sample. BHA (Benzie and Strain, 1996) used as a positive control.

c) **Total Anti-oxidant activity (TAC)[81]**

The total antioxidant capacity of *Trigonella foenum graceum* nano-phytosomes and extract was measured spectrophotometrically by the phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at 695 nm. Electron transfer occurs in this assay which depends upon the structure of the anti-oxidant. Total anti-oxidant activity was determined for *Trigonella foenum graceum* nano-phytosomes and Extract in terms of mg equivalent of ascorbic acid per g crude extract using phosphomolybdate complex assay.

### 5.2.7. *INVIVO* ANTI –DIABETIC ACTIVITY FOR *TRIGONELLA FOENUM GRACEUM* NANOPHYTOSOMES AND EXTRACT:

**a) Materials:**

Glucometer and stirps to measure blood glucose level, Gloves, Face mask, Dissection box, oral needle, sterile cloth, Lancet.,Surgical blade,Insulin syringe,Normalsaline.

**b) Animals:**

Healthy Swiss albino rats of either sex weighing 150-200g obtained from the AKCP animal house**(CPCSEA approval no: 509/02/C/CPCSEA/2018)**, housed under specific pathogen-free conditions were used for the study. The animals were placed at random and housed in polypropylene cages and were left 7 days for acclimatization to animal room maintained under controlled condition (a 12 h light–dark cycle at 22±2$^0$C and relative humidity of 30-70%). All animals were allowed to free access to water and fed with standard commercial pellet diet. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA. The Institutional Animal Ethics Committee (IAEC) approved the protocol **(AKCP/IAEC/02/18-19).** All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee .Animals were used for anti-diabetic study.

**c) Evaluation of oral glucose tolerance test (OGTT)[70]:**

Initial screening of fractions for hypoglycemic activity was carried out in normal healthy rats by conducting OGTT. The OGTT was performed for two different doses of *Trigonella foenum graceum* nanophytosomes (50 and100 mg/kg bodyweight per orally) and blood glucose level was measured by one touch glucometer (Accu-check, India). After 60 minutes of drug administration the rats were treated with 2g/kg of glucose given orally.The blood samples were collected from tail vein at the intervals of 0, 30, 60, 90 and120 min to determine the blood glucose levels.

**d) Induction of diabetes[50]:**

Diabetes was induced by a single intra-peritoneal injection of newly prepared Streptozocin(65mg/kg) in normal sterile saline to overnight fasted rats. In order to

prevent initial drug induced hypoglycemia, Streptozocin injected animals were given with 5% glucose water for 24 h. After three days of STZ administration, rats were divided according to their fasting blood glucose levels which showed >200 mg/dl. The animals did not show the above blood glucose range is excluded from the study.

**e) Grouping of animals:**

Trigonella foenum graceum leaf extract and nano-phytosomes was evaluated for diabetic activity by steptozocin induced diabetes in rat model. Thirty six animals (albino rats, 180-250 g) were divided into 6 groups of six each[51].

**Group 1:** Control received normal saline (10 ml/kg, p.o.); **Group 2:** Negative control (Diabetic rat); **Group 3:** Standard Metformin (250 mg/kg/bd.wt., orally dissolved in normal saline); **Group 4:** *Trigonella foenum graceum* nano-phytosomes (50mg/kg/bd.wt., orally); **Group 5:** *Trigonella foenum graceum* nano-phytosomes (100mg/kg/bd.wt., orally); **Group 6:** *Trigonella foenum graceum* aqueous extract (200mg/kg/bd.wt., orally)

**f) Treatment of diabetes:**

For therapy, the streptozocin induced diabetes rat model was treated once daily with oral administration of prepared *Trigonella foenum graceum* nano-phytosomes, *Trigonella foenum graceum* extract and standard drug (Metformin) for a period of 21days.

**g) Measurement of body weight and blood glucose level:**

Body weight and blood glucose levels were estimated on 0,7,14,21$^{st}$ day after the administration of extract orally. The blood glucose levels were measured using blood glucose test strips with elegance glucometer.

### 5.2.8. *INVITRO* ANTI-DIABETIC ACTIVITY:

**a) Experimental procedure for α-amylase inhibition assay[66]:**

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The

reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

The concentration of the plant extracts required to scavenge 50% of the

radicals (IC50) was calculated by using the percentage scavenging activities at five

different concentrations of the extract. Percentage inhibition (I %) was calculated by

I % = (AC-AS)/ AC x 100

Where,

Ac = Absorbance of the control

As = Absorbance of the sample

**b) α-Glucosidase Inhibitory Assay[66]:**

The effect of the plant essential oil on α-glucosidase activity was determined according to the method described by Kim et al., [12], using α-glucosidase from Saccharomyces cerevisiae. The substrate solution p-nitropheynyl glucopyranoside (pNPG) (3.0 mM) was prepared in 20 mM phosphate buffer, pH 6.9. 100 µL of α-glucosidase (1.0 U/ml) was pre-incubated with 50 µL of the different concentrations of the extracts (ethanol) for 10 mins. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added. to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 ml of 0.1 M Na2CO3 The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from pNPG at 405 nm. The results (% Inhibition) are expressed as percentage of the blank.

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

I % = (AC-AS)/ AC x 100

Where,

Ac = Absorbance of the control

As = Absorbance of the sample

### 5.2.9. Biochemical studies

At the end of 21st day treatment, rats were sacrificed by phenobarbitone anaesthesia and blood samples are collected and used for the Bio-chemical assay, Histopathological studies, etc.

**Preparation of Serum**

Blood was collected by cardiac puncture. Blood was allowed to flow freely into plain bottles. The serum was prepared using standard method as described by Yesufu et al.,[51]. Briefly, the method used is as follows. Blood was allowed to clot for 30minutes and then centrifuged at 2500rpm for 15minutes and serum was harvested.

The Bio-chemical estimation which have done are,

a. Estimation of SuperOxide Dismutase activity (SOD)
b. Estimation of Catalase activity (CAT)
c. Estimation of Reduced Glutathione (GST)
d. Thio Barbituric Acid Reactive Substance (TBARs)

**a) Estimation of Superoxide Dismutase activity (SOD)[82]**

Superoxide dismutase activity was assayed according to the method of Sun et al. In this method, xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of enzyme providing 50% inhibition of NBT reduction. Results are expressed as U/mL.

**b) Estimation of Catalase Activity (CAT)[83]**

The catalase activity in serum was determined using the modified method as described by Atawodi. Briefly, the method is as follows: serum (10 was added to test tube containing 2.80mL of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1mL of fresh 30mM hydrogen peroxide and the decomposition rate of hydrogen peroxide was measured at 240nm for 5min on a spectrophotometer. A molar extinction coefficient of 0.041mM−1 cm−1 was used to calculate catalase activity.

**c) Estimation of Reduced glutathione (GSH)[84]**

Equal quantity of serum was mixed with 10% trichloroacetic acid and the mixture was centrifuged to separate proteins. To, 0.01ml of this supernatant, 2ml of phosphate buffer (pH – 8.4). 0.5ml of 5'5 dithiobis (2-nitrobenzoic acid) and 0.4 ml of distilled water was added. Mixture was vortexed glutathione was expressed as µg/mg of proteins.

**d) Thio Barbituric Acid Reactive Substance (TBARS)[85]**

The Thio Barbituric Acid reactive Substance (TBARS) was estimated as per the standard procedure. To each test tube , 0.5 ml of supernatant, 0.5 ml of normal saline, 1ml of 20% trichloro acetic acid (TCA) and 0.25ml of TBA reagent (200 mg of thiobarbituric acid in 30ml of distilled water and 3oml of acetic acid) were added. The test tubes were kept for boiling at 950c for 1 hour. To each test tube, 3ml of n-butanol was added and mixed well. These test tubes were centrifuged at 3000rpm for 10mis. The separated butanol layer was collected and read in a spectrophotometer against blank at 535nm. Concentration of Thio barbituric reactive substance was expressed in terms of nmol malondialdehyde per mg of protein.

**5.2.10. Histopathological study[86]:**

After blood collection rats were sacrificed for tissue studies. The internal organ pancreas were isolated and blotted free of blood, weighed immediately to determine relative organs weights and observed for gross lesions. Histological examination was performed on the tissue preserved in 10% buffered formalin solution with particular emphasis on those which showed gross pathological changes.

### 5.2.11. Statistical analysis

Results are presented as the mean ± SEM for n = 6 rat/group. All experiments were performed in triplicate using a minimum of three replicates. Values are expressed as mean ± S.E.M. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test using Graph pad prism. The values are ****P<0.0001, ***P<0.0005 were considered significant, **P<0.001 when compared against control.

# CHAPTER – VI

# RESULTS AND DISCUSSION

# CHAPTER VI
# RESULTS AND DISCUSSION

## 6.1. a. PRELIMINARY PHYTOCHEMICAL INVESTIGATION

The preliminary phytochemical studies reveals that the presence of phenols, tannins, flavanoids, and saponins **(Table 7)**. Many plants are now used for the traditional treatment of diseases, possibly due to the presence of these phytochemicals, which have been reported to possess high medicinal value. Phenols, flavonols, proanthocyanidins and flavonoids are very important plant bioactive component reported to possess strong antioxidant, antibacterial and numerous biological activities. Due to their molecules structures reported to contain a hydroxyl group or phenolics ring several studies have indicated that these compounds are very effective in scavenging free radicals due to their redox properties, thereby having the capacity to link with proteins and bacterial membranes to form complexes.

**Table 7: Phytoconstituents of Trigonella foenum graceum**

| S.NO | TEST | RESULT |
|---|---|---|
| **1.** | **TEST FOR CARBOHYDRATES** | |
| | A . Molisch’s test | + |
| | B. Fehling’s test | + |
| | C. Barfoed's test | + |
| | D. Benedict's test | + |
| **2.** | **TEST FOR PROTEINS AND AMINO ACIDS** | |
| | A. Ninhydrin Test | + |
| | B. Biuret Test | + |
| | C. .Millon’s Test | + |
| **3.** | **TEST FOR ALKALOIDS** | |
| | A. Mayer's test | - |
| | B. Dragendroff's test | - |
| | C. Wagner's test | - |

| | | |
|---|---|---|
| **4.** | **TEST FOR PHENOLIC COMPOUNDS AND TANNINS** | |
| | A. Braemer's test | + |
| **5.** | **TEST FOR TERPENOIDS** | + |
| **6.** | **TEST FOR FLAVONOIDS** | |
| | A. Shinoda test | + |
| **7.** | **TEST FOR SAPONINS** | |
| | A**.** Foam test/ Frothing test | + |
| **8.** | **TEST FOR GLYCOSIDES** | |
| | A. Legal's Test | + |
| | B. Borntrager's Test | + |
| **9.** | **TEST FOR PHYTOSTEROLS** | |
| | A..Libermann-Burchard test | - |
| | B.Salkowski reactions | - |
| **10** | **TEST FOR GUM MUCILAGE** | + |

**(+) indicates positive reaction (-) indicates negative reaction**

### 6.1. B.THIN LAYER CHROMATOGRAPHY

To support phytochemical screening, aqueous extract leaves of *Trigonella foenum graceum* was subjected to thin layer chromatography. The *Trigonella foenum graceum* extract showed two well separated spot in the mobile phase Methanol : Chloroform (1:9). TLC findings were in agreement with the data of qualitative chemical tests and the spots characteristic for flavonoids were observed. The Rf values of the spots from *Trigonella foenum graceum* were calculated and found in iodine vapour as as 0.66 (Figure 7)

$R_f$ value of aqueous extract of *Trigonella foenum graceum*

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$$= \frac{3.3}{5}$$

$$= 0.66$$

From the present study it is presumed as these compounds might be responsible for anti-diabetic activity of plant *Trigonella foenum graceum.*

**Figure 7 Thin layer chromatography of *Trigonella foenum graceum* extract**

## 6.2. PREPARATION AND EVALUATION OF *TRIGONELLA FOENUM GRACEUM* NANO-PHYTOSOMES

### 6.2.1. Preparation of phytosomes

Thin layer lipid hydration method was used. F1 formulation having Egg lecithin : Cholesterol : *Trigonella foenum graceum* extract in the ratio 1:0.5:1 was selected as optimized formulation on the basis of morphology and particle size and then evaluated further.

### 6.2.2. EVALUATION OF *TRIGONELLA FOENUM GRACEUM* NANO-PHYTOSOMES

#### a. Scanning Electron Microscope analysis (SEM)

Scanning electron microscopy give important insight into the solid state properties and surface morphology of drug and drug complexes. SEM analysis as presented in Figure 8 confirms the vesicle size measured by size analyzer i.e. around 100-600 nm. The drug particles are associated with the phospholipid forming complexes with spherical shape, uniform, regular and rigid vesicles.

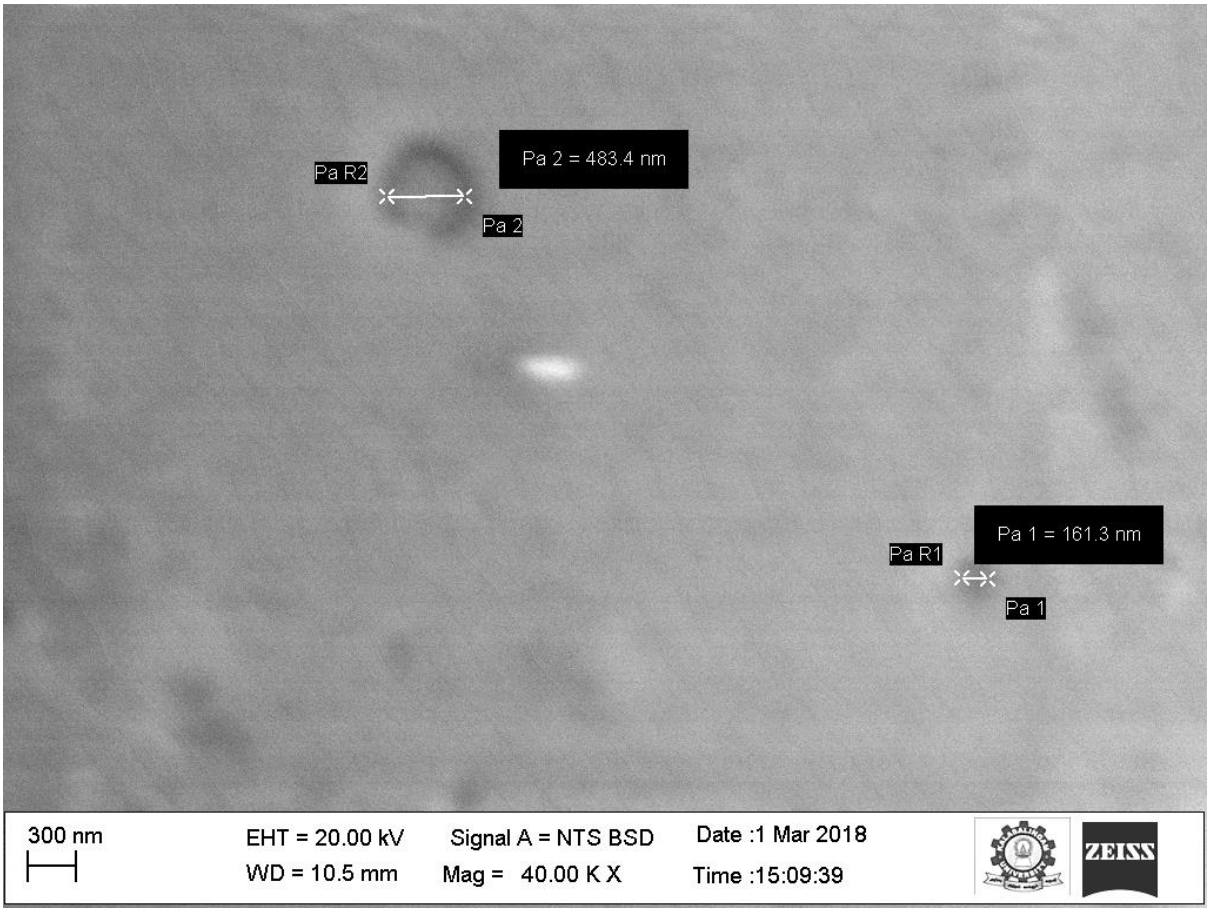


**Figure 8 SEM of *Trigonella foenum graceum* nano-phytosome**

## b. Differential scanning calorimetry analysis (DSC)

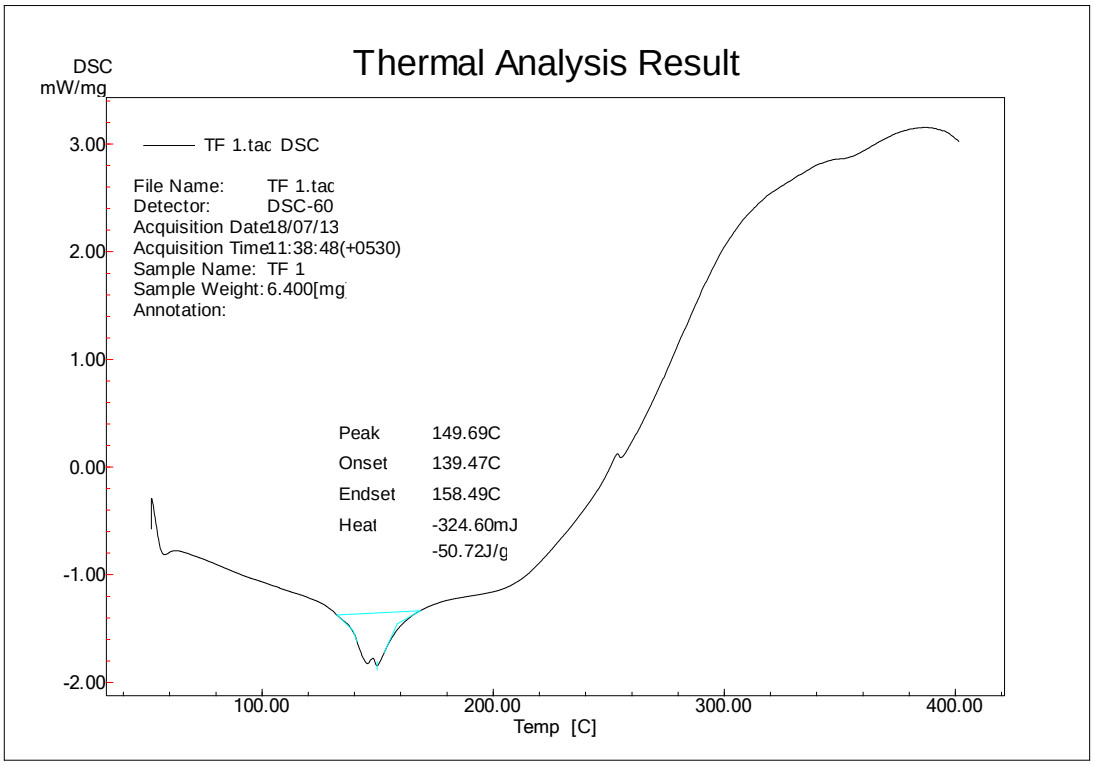


**Figure 9 S-I DSC *Trigonella foenum graceum* thermogram of extract**

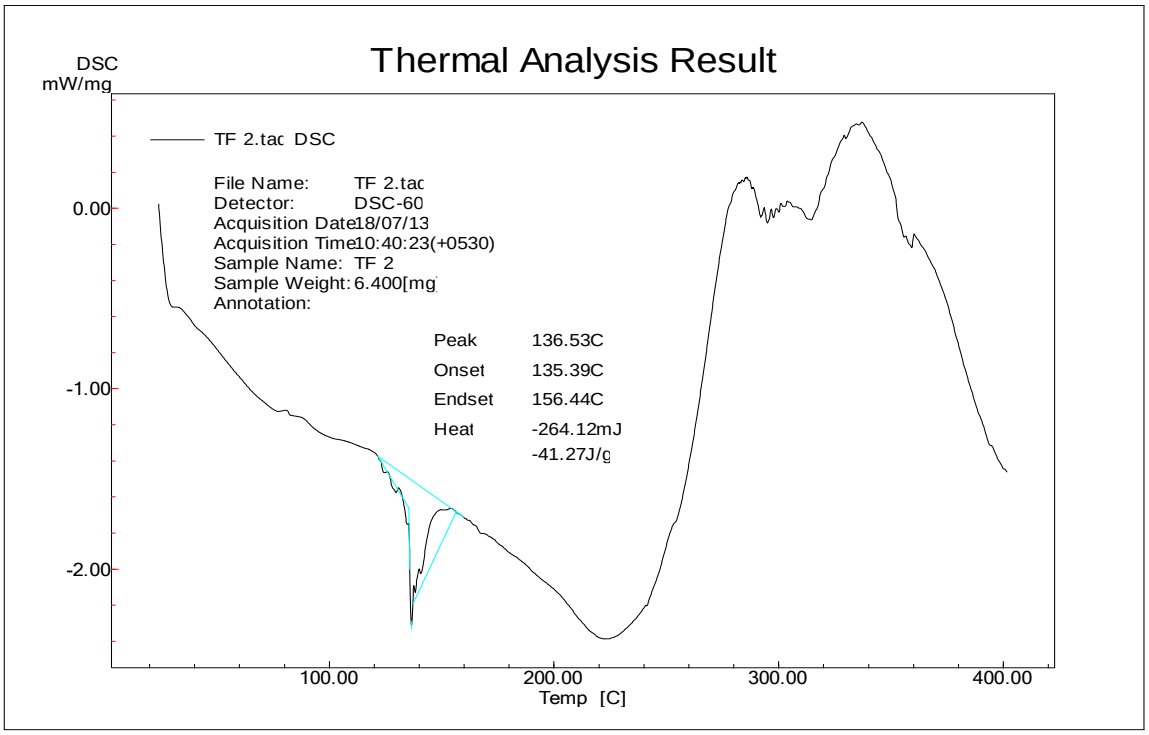


**Figure 10 S-II DSC thermogram of Egg lecithin and Cholesterol**

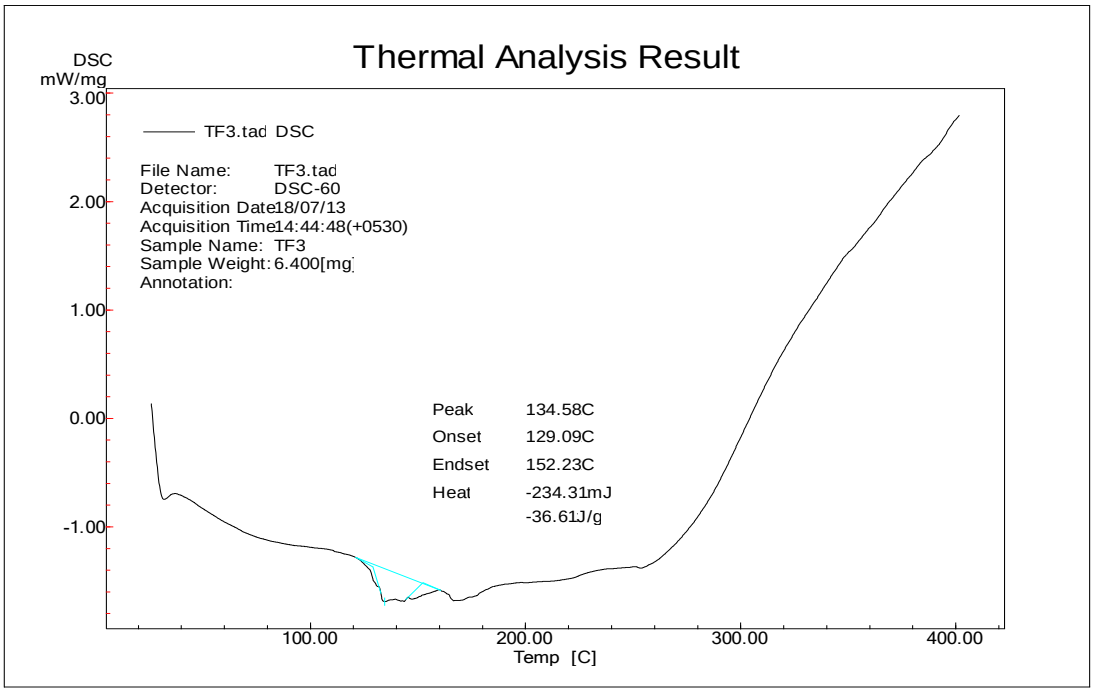


**Figure 11 S-III DSC thermogram of physical mixtures of *Trigonella foenum graceum* extract , egg lecithin, cholesterol**

Differential scanning calorimetry study was carried out to ensure formation of complex between *Trigonella foenum graceum* extract, cholesterol and egg lecithin, to confirm the presence of *Trigonella foenum graceum* extract in the phytosome. DSC thermograms of the pure *Trigonella foenum graceum* extract, cholesterol and egg lecithin, physical mixture of them are shown in Figure 9,10,11 . DSC thermograms of Pure *Trigonella foenum graceum* extract (S I) showed a endothermal peak at 149.69$^0$C and its beginning melting point was 139.47$^o$c, it is attributed to its melting point. Egg lecithin and Cholesterol (S II) showed an endothermal peak at 136.53$^o$c. DSC thermogram of physical mixture of *Trigonella foenum graceum* extract, cholesterol, egg lecithin (S III) showed a endothermal peak at 134.58$^o$C. The carbon-hydrogen chain in soya lecithin perhaps happened to be melt, isomerous or the crystal changes. The physical mixture of *Trigonella foenum graceum* extract, Cholesterol and Egg lecithin showed a broad endothermal peak at 134.58$^o$c. When compare the *Trigonella foenum graceum* extract (149.69$^0$C) with Physical mixture (134.58$^o$C) it showed that there is no wide variation between the peaks and the difference is within ±20$^o$C. This slight variation in the peaks may be due to the physical interact by Hydrogen bonding between-OH group of *Trigonella foenum graceum* extract and polar part of phosphatidylcholine suggested by Beggan et.al., (1999). On the other hand, in the *Trigonella foenum graceum* extract–cholesterol-egg lecithin complex, the major endothermic peak was observed at 134.58 °C, which was different from the peaks of the individual components of the complex. Thermogram of Physical mixtures of *Trigonella foenum graceum* extract, egg lecithin and cholesterol interestingly showed disappearance of the endothermic melting peak of *Trigonella foenum graceum* extract, indicating that *Trigonella foenum graceum* was completely embedded inside the matrix of physical mixture which had different thermal properties. The results are depicted in Figure 9,10,11.Hence,DSC studies conformed the absence of drug-excipient interactions.

## c. Fourier Transform Infra Red Spectroscopic analysis (FT-IR)

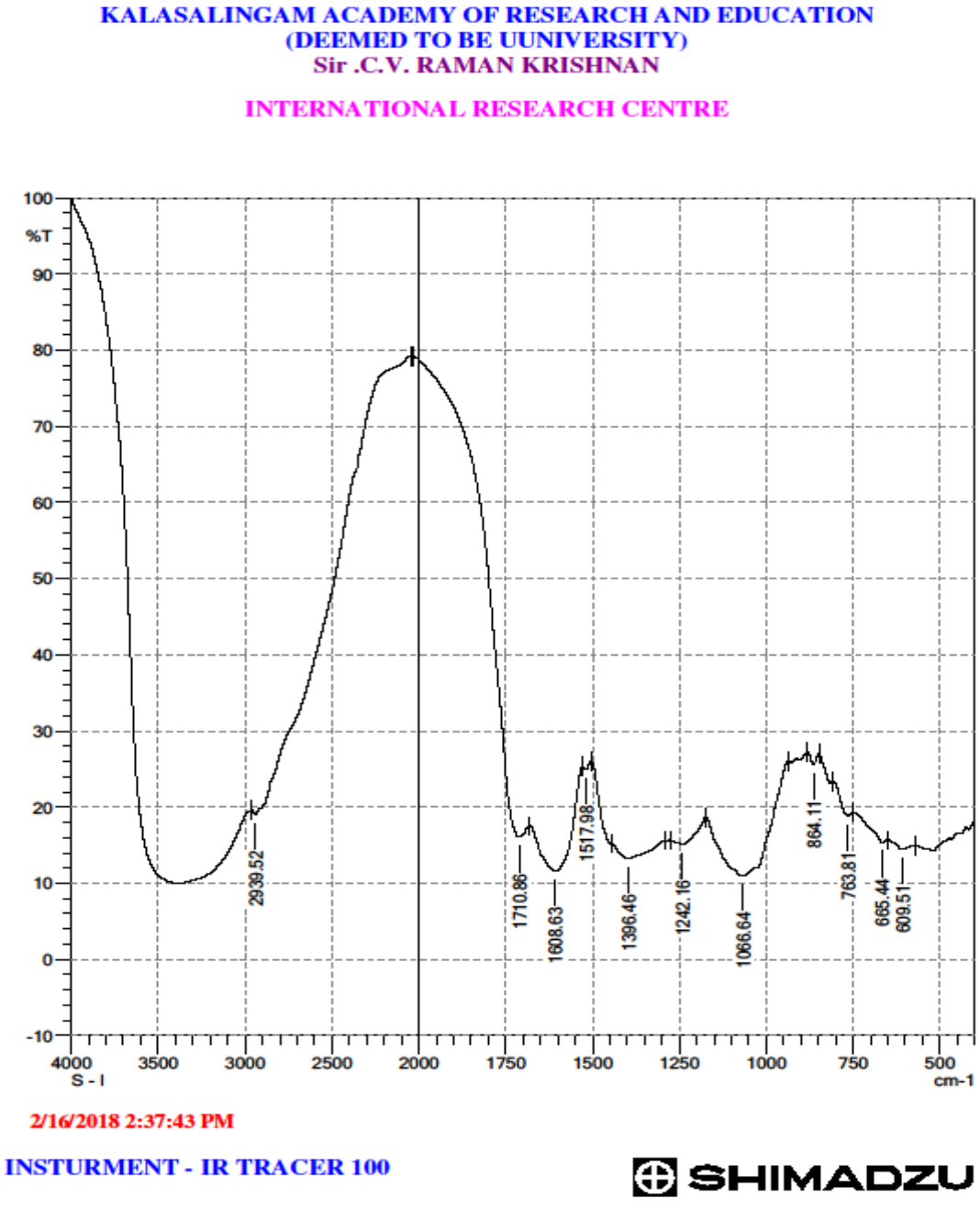


**Figure 12 S-I represents FT-IR spectra of Trigonella foenum graceum extract**

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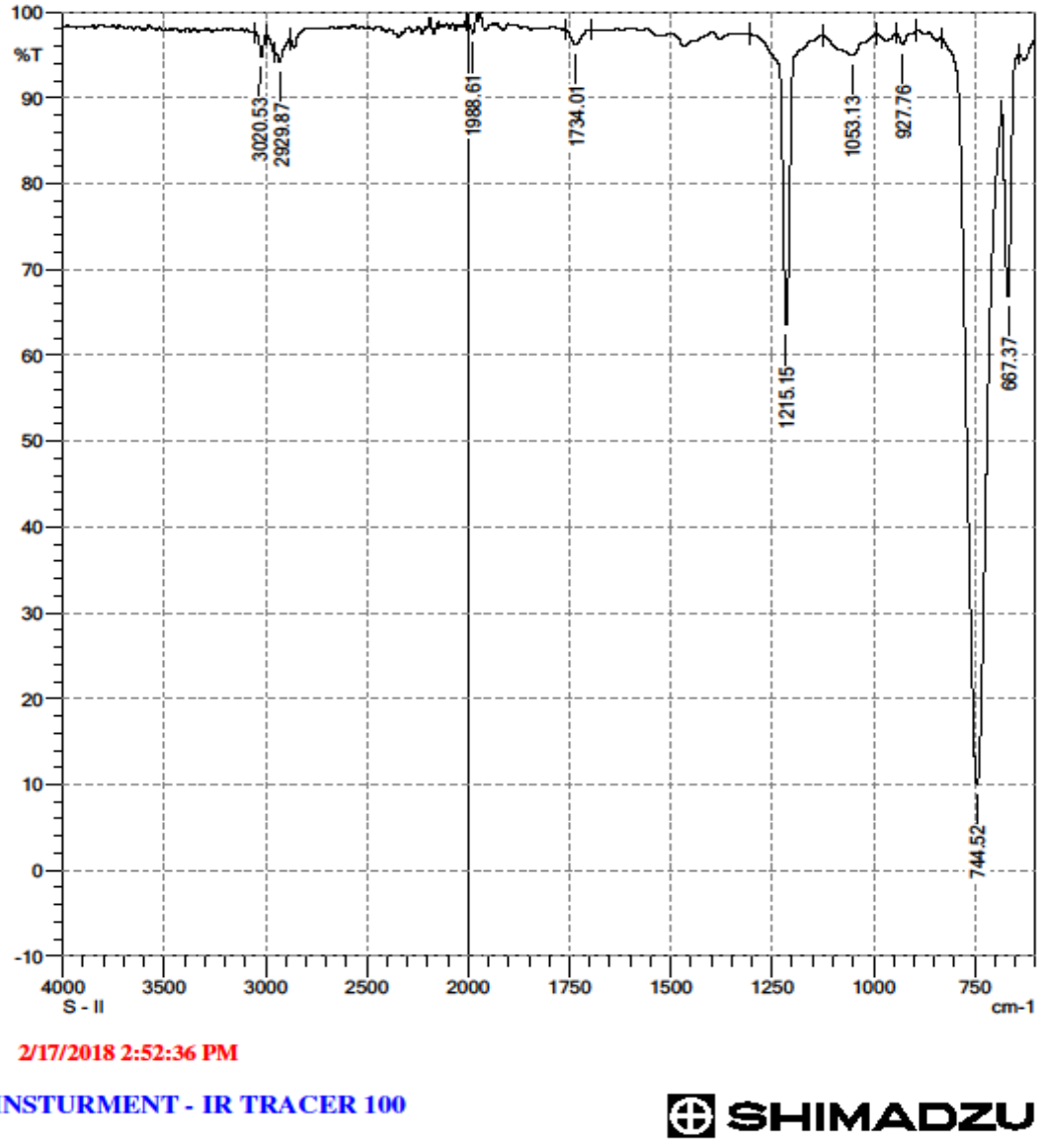


**Figure 13 S-II FT-IR spectra of Physical mixture of egg lecithin and cholesterol**

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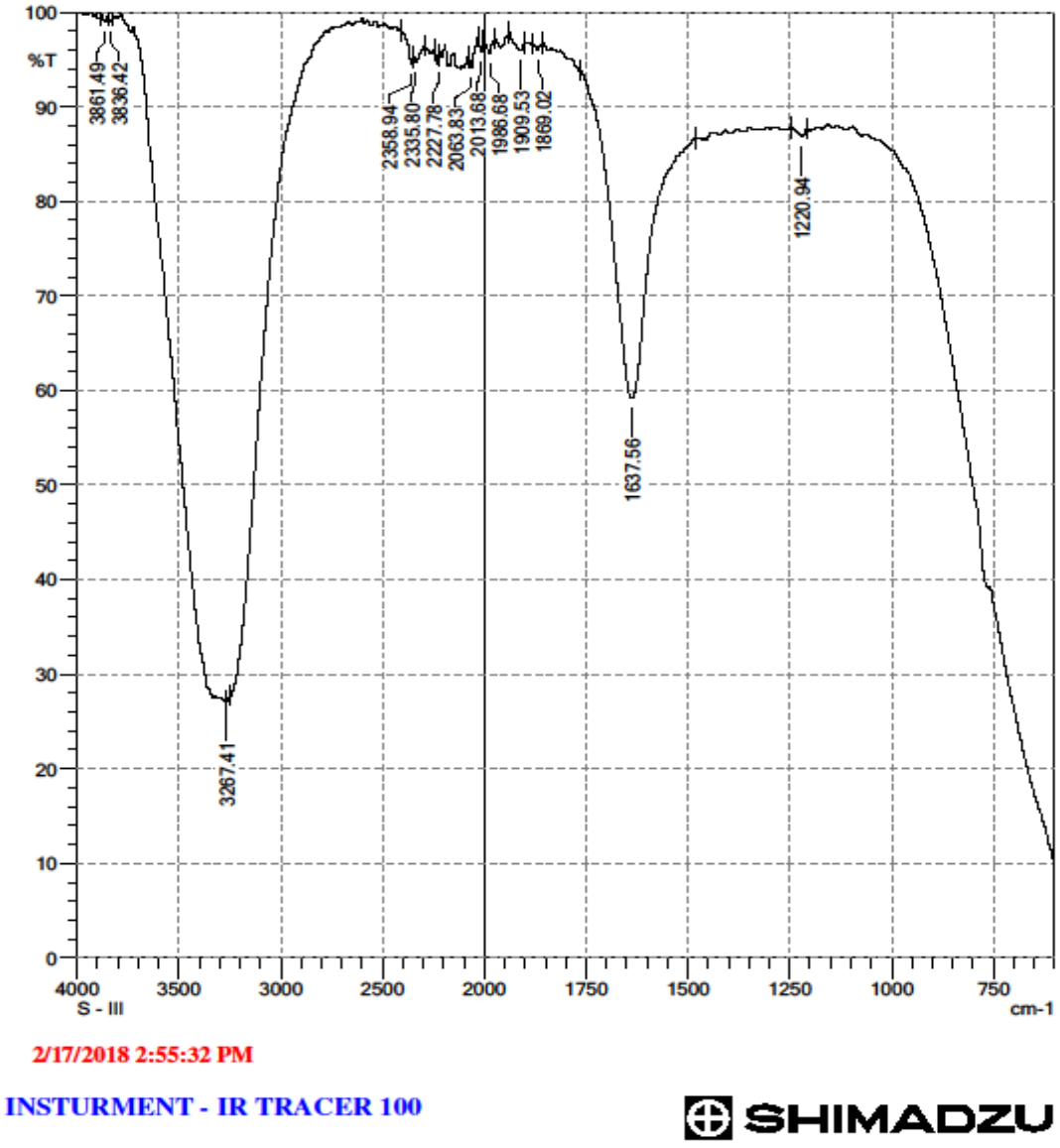


**Figure 14 S-III Trigonella foenum graceum nano-phytosome**

Spectroscopic analysis was used in order to identify and diagnose of complex formation between phospholipid and *Trigonella foenum graceum* extract. In FT-IR spectroscopy, functional groups and their numbers were identified from the frequency of radiation that absorbs infrared spectra which showed the main chemical groups in extract and PC as well as the formation of new interactions between them in the nano-phytosomes preparation process (Table 8). The FT-IR spectroscopy of *Trigonella foenum graceum* leaf extract (S-I), mixture of Cholesterol and egg lecithin (S II), *Trigonella foenum graceum* nano-phytosome (S-III) are shown figure 12, 13, 14. *Trigonella foenum graceum* leaf extract (S-I) shows that the characterstic C-H peak at 2939.52 $cm^{-1}$, C=O peak at 1710.86 $cm^{-1}$, C-C peak at 1608.63 $cm^{-1}$, C-C peak at 1517.98 $cm^{-1}$, C-H peak at 1396.46 $cm^{-1}$, C-N peak at 1242.16 $cm^{-1}$, C-N peak at 1066.64 $cm^{-1}$. *Trigonella foenum graceum* nano-phytosomes (S-III) shows that the characteristic peak at O-H peak at 3861.49 $cm^{-1}$, O-H peak at 3836.42 cm-1, N-H peak at 3267.41 $cm^{-1}$, C≡ N peak at 2358.94 $cm^{-1}$, C≡ N peak at 2335.80 $cm^{-1}$, C≡ N peak at 2227.78 $cm^{-1}$, C=C peak at 2013.68 $cm^{-1}$, C=C peak at 1986.68 $cm^{-1}$,C=C peak at 1909.53 $cm^{-1}$, C=C peak at 1869.02 $cm^{-1}$, N-H peak at 1637.56 $cm^{-1}$, C-H peak at 1453.16 $cm^{-1}$, C-C peak at 1412.23 $cm^{-1}$, C-C peak at 1398.76 $cm^{-1}$, C-H peak at 1357.17 $cm^{-1}$, N-O peak at 1346.78 $cm^{-1}$, C-N peak at 1220.94 $cm^{-1}$. Some of the peaks of both *Trigonella foenum graceum* extract and phospholipid were shown at their physical mixture spectrum indicating no chemical interaction was occurred in this physical mixture.

**Table 8 Interpretation of FT-IR studies**

| S.No. | Functional Group | Wave number ($cm^{-1}$) | | | |
|---|---|---|---|---|---|
| | | Reference | *Trigonella foenum graceum* Extract | Phospholi-pid mixture | *Trigonella foenum graceum* nano-phytosome |
| 1 | O-H (stretch) Free hydroxyl, alcohols, phenols | 3640-3610 | | | 3861.49 |

| | | | | | |
|---|---|---|---|---|---|
| 2 | O-H(stretch) Free hydroxyl, alcohols, phenols | 3640-3610 | | | 3836.42 |
| 3 | N-H(stretch) $1^0$,$2^0$ amines,amides | 3400-3250 | | | 3267.41 |
| 4 | C-H(stretch) aromatics | 3100-3000 | | 3020.53 | |
| 5 | C-H(stretch) alkanes | 3000-2850 | 2939.52 | 2929.87 | |
| 6 | CΞN(stretch) nitriles | 2260-2210 | | | 2358.94 |
| 7 | CΞN(stretch) nitriles | 2260-2210 | | | 2335.80 |
| 8 | CΞN(stretch) nitriles | 2260-2210 | | | 2227.78 |
| 9 | C=C(stretch) alkenes | 1900-2000 | | | 2013.68 |
| 10 | C=C(stretch) Alkanes | 1900-2000 | | 1988.61 | 1986.68 |
| 11 | C=C(stretch) alkenes | 1900-2000 | | | 1909.53 |
| 12 | C=C(stretch) alkenes | 1900-2000 | | | 1869.02 |

| | | | | | |
|---|---|---|---|---|---|
| 13 | C=O(stretch) aldehyde, ketones, saturated aliphatic | 1665-1740 | 1710.86 | 1734.01 | |
| 14 | C-C(stretch) Aromatics | 1600-1585 | 1608.63 | | 1637.56 |
| 15 | C-C(stretch) Aromatics | 1500-1400 | 1517.98 | | 1398.76 |
| 16 | C-H(Bend) alkanes | 1470-1450 | | | 1453.16 |
| 17 | C-C(stretch) Aromatics | 1500-1400 | | | 1412.23 |
| 18 | C-H(rocking) Alkanes | 1370-1350 | 1396.46 | | 1357.17 |
| 19 | N-O Symmetric (stretch) nitro compounds | 1360-1290 | | | 1346.78 |
| 20 | C-N(stretch) Aliphatic amines | 1250-1020 | 1242.16 | 1215.15 | 1220.94 |
| 21 | C-N(stretch) Aliphatic amines | 1250-1020 | 1066.64 | 1053.13 | |

Hence,FTIR studies further supported the absence of incompatability between drug and excipients.

**d. Particle size (PS)**

2018.02.22 15:50:09

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HORIBA SZ-100 for Windows [Z Type] Ver2.10

## SZ-100

### Measurement Results

| | |
|---|---|
| Date | : Thursday, February 22, 2018 3:43:00 PM |
| Measurement Type | : Particle Size |
| Sample Name | : S1 |
| Scattering Angle | : 173 |
| Temperature of the Holder | : 25.0 °C |
| Dispersion Medium Viscosity | : 0.895 mPa·s |
| Transmission Intensity before Meas. | : 966 |
| Distribution Form | : Standard |
| Distribution Form(Dispersity) | : Monodisperse |
| Representation of Result | : Scattering Light Intensity |
| Count Rate | : 1725 kCPS |

### Calculation Results

| Peak No. | S.P.Area Ratio | Mean | S. D. | Mode |
|---|---|---|---|---|
| 1 | 1.00 | 311.6 nm | 81.8 nm | 295.7 nm |
| 2 | — | — nm | — nm | — nm |
| 3 | — | — nm | — nm | — nm |
| Total | 1.00 | 311.6 nm | 81.8 nm | 295.7 nm |

### Cumulant Operations

| | |
|---|---|
| Z-Average | : 251.5 nm |
| PI | : 0.708 |

### Molecular Weight Measurement

| | |
|---|---|
| Molecular Weight | : — |
| Parameters for Molecular Weight Calculation | : — |

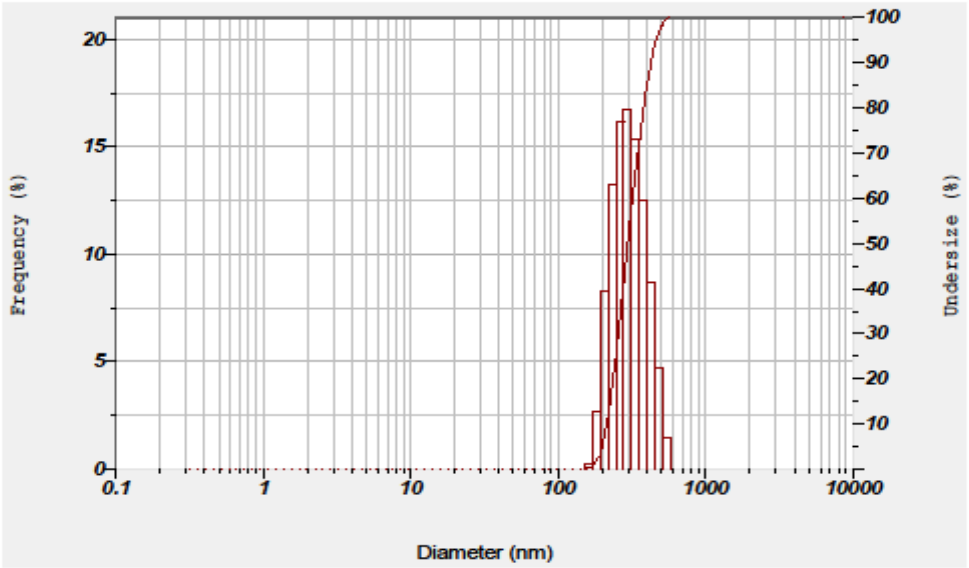


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**Figure 15 Particle size of *Trigonella foenum graceum* nano-phytosomes**

Particle size plays an important role in the stability, availability and organoleptic properties of the Nanophytosomal formulation and the particles with smaller size is desirable. Results of particle size analysis indicated that nano-phytosomes prepared with *Trigonella foenum graceum* extract and PC possess the particle size in the average range of 251nm with polydispersity index of 0.708 (Figure 15). Vesicle size tends to increase with increasing concentration of the complex. When the concentration of particles is too high, physical interaction either collision or electrostatic between vesicles is more pronoun. These interactions alter the movement of the particles and produce vesicles with a larger size. The high lipid composition in the formulation also increases the tendency for the formation of agglomerates, resulting in the bigger size of the vesicles. This indicates the uniformity and homogeneity of the size of vesicles in the system. A relative high polydispersity index was observed in *Trigonella foenum graceum* nano-phytosomes which may be attributed to high phospholipid content.

**e. Zeta Potential(ZP)**

2018.02.22 15:59:45

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HORIBA SZ-100 for Windows [Z Type] Ver2.10

SZ-100

## Measurement Results

### Measurement Results

| | | |
|---|---|---|
| Date | : | Thursday, February 22, 2018 3:53:35 PM |
| Measurement Type | : | Zeta Potential |
| Sample Name | : | S 1 |
| Temperature of the Holder | : | 25.0 °C |
| Dispersion Medium Viscosity | : | 0.895 mPa·s |
| Conductivity | : | 0.163 mS/cm |
| Electrode Voltage | : | 3.4 V |

### Calculation Results

| Peak No. | Zeta Potential | Electrophoretic Mobility |
|---|---|---|
| 1 | -38.6 mV | -0.000299 cm2/Vs |
| 2 | — mV | — cm2/Vs |
| 3 | — mV | — cm2/Vs |

Zeta Potential (Mean) : -38.6 mV

Electrophoretic Mobility Mean : -0.000299 $cm^2/Vs$

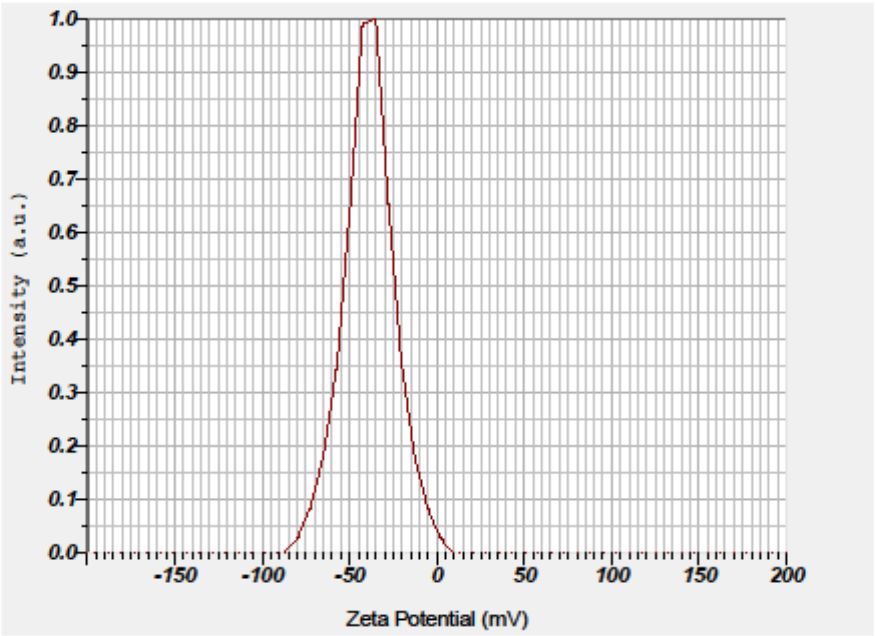


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**Figure 16 Zeta potential of Trigonella foenum graceum nano-phytosomes**

The Zeta potential is the electric potential in the interface or particle surface and is used to predict the stability of colloidal systems. The magnitude of the Zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles which affects the stability of colloidal system. Colloids with high absolute Zeta potential values (normally ±30 mV), regardless of their positivity or negativity, are electrically stabilized and those with low Zeta potential values are not stable and tend to coagulate or flocculate. In general, higher Zeta potential values indicate a higher and longer-term stability of the particles. Several factors such as pH, ionic strength, type and concentration of the used biopolymers are effective on the Zeta potential of the particles. The surface charge analysis results ( -38.6 mV) are shown in (Figure 16 ) and point to the physical stability of *Trigonella foenum graceum* nano-phytosomes.

**f)Transmission Electron Microscope (TEM):**

TEM analysis as presented in confirmed the spherical shape of the vesicle. The aggregation between vesicles was observed in single particle shape, forming irregular large size particles. TEM pictures depicted that the nanophytosomes are discretely distributed. Nanophytosomes are nearly spherical in shape with smooth surfaces. TEM picture(Figure 17) shows the internal architecture of the nanophytosomes.

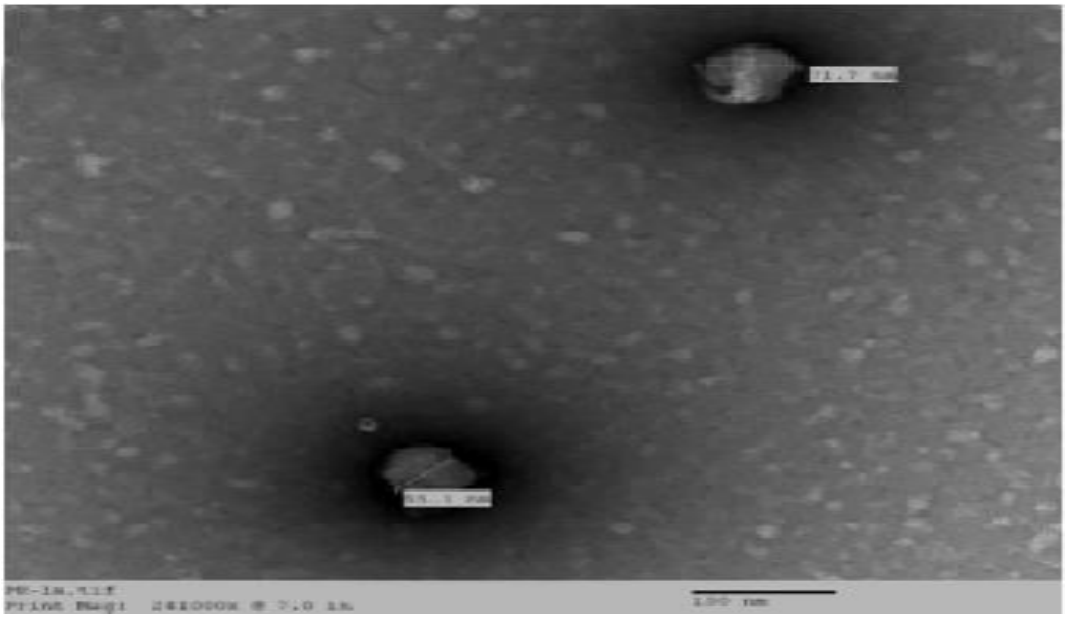

**Figure 17 TEM analysis of *Trigonella foenum-graecum* nano-phytosomes**

**F. X-Ray diffaction studies of nanophytosomes**

Presence of single peak at $20^0$ of 2theta indicates lack of crystalinity of the nanophytosomes and the absence of sharp peaks inferes the drug become amorphous after nanonization process shown in figure 18

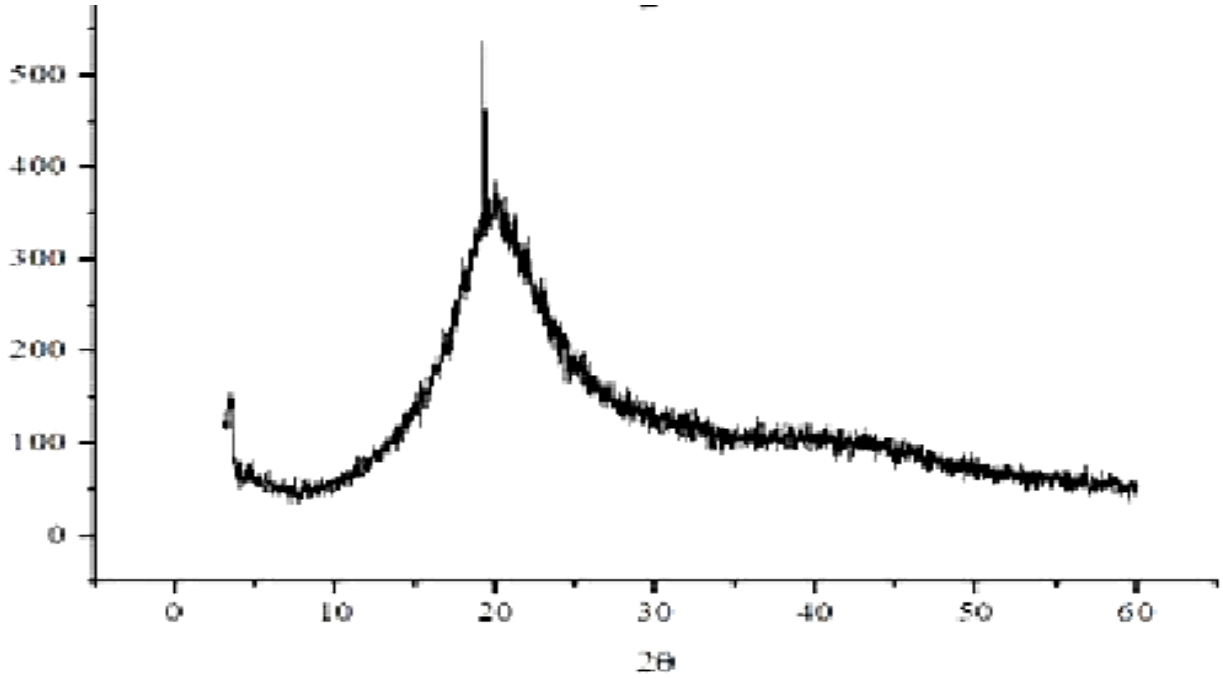


**Figure 18 XRD analysis of *Trigonella foenum-graecum*nano-phytosomes**

## 6.3. SCREENING OF *IN VITRO* ANTI-OXIDANT ACTIVITY OF *TRIGONELLA FOENUM GRACEUM* NANO-PHYTOSOMES AND EXTRACT

### 6.3.1. Nitric oxide radical scavenging activity

Nitric oxide is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and anti tumour activities. Suppression of released NO may be partially attributed to direct NO scavenging, as the *Trigonella foenum graceum* nano-phytosomes decreased the amount of nitrite generated from the decomposition of SNP in vitro. The scavenging of NO by the *Trigonella foenum graceum* nano-phytosomes was increased in concentration dependent manner. Figure 19 illustrates a significant decrease in the NO radical due to the scavenging ability of nano-phytosomes, extract and BHA(Standard Antioxidant). The *Trigonella foenum graceum* nano-phytosome showed maximum activity of 38.42% at 250µg/ml, whereas BHA at the same concentration exhibited 41.04% inhibition (Table 9).

**Table 9 Nitric oxide radical scavenging activity of *Trigonella foenum graceum* nano-phytosome and extract**

| S.No. | Concentration (µg/ml) | % of scavenging of Nitric oxide radical | | |
|---|---|---|---|---|
| | | BHA(Butylated hydroxy Anisole) | *Trigonella foenum graceum* nano-phytosome | *Trigonella foenum graceum* extract |
| 1 | 50 | 21.17±0.36 | 19.03±0.29** | 12.96±0.23**** |
| 2 | 100 | 24.85±0.37 | 21.37±0.33** | 16.23±0.42**** |
| 3 | 150 | 31.99±0.44 | 26.65±0.27**** | 20.69±0.24**** |
| 4 | 200 | 36.84±0.22 | 31.96±0.26**** | 25.36±0.32**** |
| 5 | 250 | 41.04±0.46 | 38.42±0.27** | 31.24±0.20**** |

Values are expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. The values are ****P<0.0001, ***P<0.0005, **P<0.001 when compared against control.

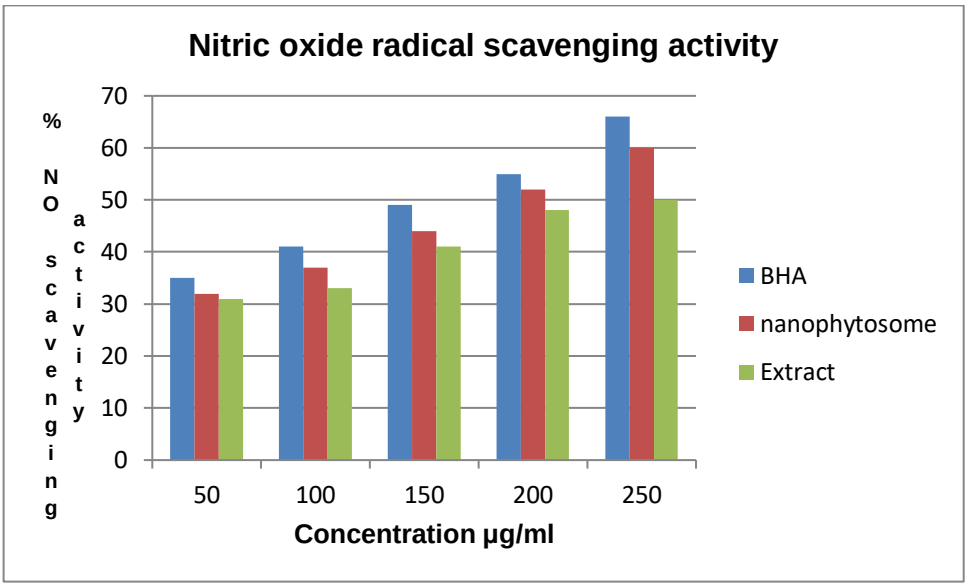


**Figure 19 represents Nitric oxide radical scavenging activity**

### 6.3.3. Ferric reducing Anti-oxidant power assay (FRAP)

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe3+-TPTZ] complex and producing a coloured ferrous tripyridyltriazine [Fe2+-TPTZ]11. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. Frap assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction. In the present study, the trend for ferric ion reducing activities of *Trigonella foenum graceum* nano-phytosomes, extract and BHA are shown in Figure 20 and table 10 . The absorbance of *Trigonella foenum graceum* nano-phytosomes are clearly increased, due to the formation of the Fe2+-TPTZ complex with increasing concentration. The *Trigonella foenum graceum* nano-phytosmes and *Trigonella foenum graceum* extract showed increased ferric reducing power with the increased concentration as standard antioxidant. Hence they should be able to donate electrons to free radicals stable in the actual biological and food system.

**Table 10 represents FRAP assay of *Trigonella foenum graceum* nano-phytosome and extract**

| S.No. | Concentration µg/ml | OD at 593 nm | | |
|---|---|---|---|---|
| | | BHA | *Trigonella foenum graceum* nano-phytosome | *Trigonella foenum graceum* extract |
| 1 | 100 | 0.351±0.004 | 0.323±0.003** | 0.312±0.004*** |
| 2 | 200 | 0.417±0.004 | 0.377±0.003*** | 0.332±0.004**** |
| 3 | 300 | 0.490±0.003 | 0.446±0.006*** | 0.414±0.004**** |
| 4 | 400 | 0.550±0.003 | 0.525±0.004** | 0.480±0.003**** |
| 5 | 500 | 0.660±0.024 | 0.606±0.006 | 0.505±0.004*** |

Values are expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. The values are ****P<0.0001, ***P<0.0005, **P<0.001 when compared against control.

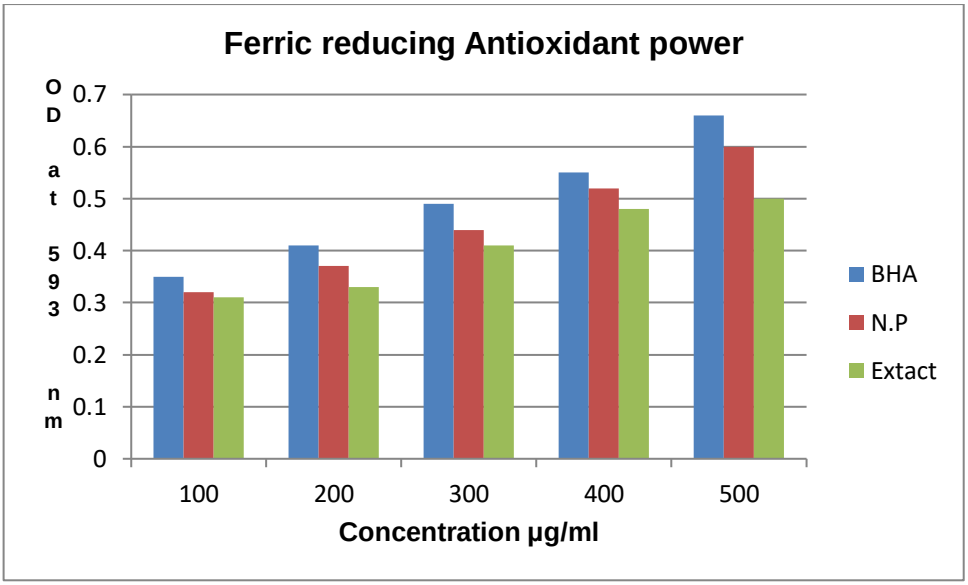


**Figure 20 Ferric reducing Antioxidant power**

### 6.3.4. Total Anti-Oxidant activity

Total anti-oxidant activity using phosphomolybdate complex assay of *Trigonella foenum graceum* crude extract and nano-phytosome was determined and expressed in ascorbic acid equivalent per gram of extract and nano-phytosome.

**Table 11 represents Total Anti-Oxidant activity of *Trigonella foenum graceum* nano-phytosome and Extract**

| | |
|---|---|
| Total Anti-oxidant activity µ mole of Ascorbic acid equivalent per g crude extract of *Trigonella foenum graceum* | 171.12±3.56 |
| Total Anti-oxidant activity µ mole of Ascorbic acid equivalent per ml of *Trigonella foenum graceum* Nano-phytosome | 234.67±2.38 |

Values are expressed as mean ± S.E.M.

## 6.4.EVALUATION OF *INVIVO* ANTI-DIABETIC ACTIVITY

### 6.4.1. Effect of *Trigonella foenum graceum* nanophytomes on Oral Glucose Tolerance Test(OGTT) in experimental rats:

In ogtt test investigation group 1 served as control, group 2 served as *Trigonella foenum graceum* nanophytomes 50mg/kg body weight and group 3 served as *Trigonella foenum graceum* nanophytomes 100mg/kg body weight.The blood levels of glucose were demonstrated the significant change after oral administration of glucose solution.Dose dependent blood glucose reduction was observed in rats(group2 and group 3)after treatment with *Trigonella foenum graceum* nanophytomes 50 and 100mg/kg at 0,60,90and 120 min The results are given below in the table 12 and figure:21

**Table 12 represents oral glucose tolerance test in rats**

| Treatment | Dose | Blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| **Group I** | Normal saline 0.9% Nacl | 76.54±1.45 | 122.78±1.87*** | 137.34±1.46*** | 112.66±1.05** | 73.54±3.56 |
| **Group II** | 50 mg/kg | 72.56±0.96 | 112.51±1.58** | 132.48±1.42*** | 101.22±1.07** | 70.42±2.42 |
| **Group III** | 100 mg/kg | 78.74±1.39 | 101.87±1.43 | 120.66±0.58** | 91.43±1.68 | 67.38±2.19 |

Values are expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. The values are ****P<0.0001, ***P<0.0005, **P<0.001 when compared against control.

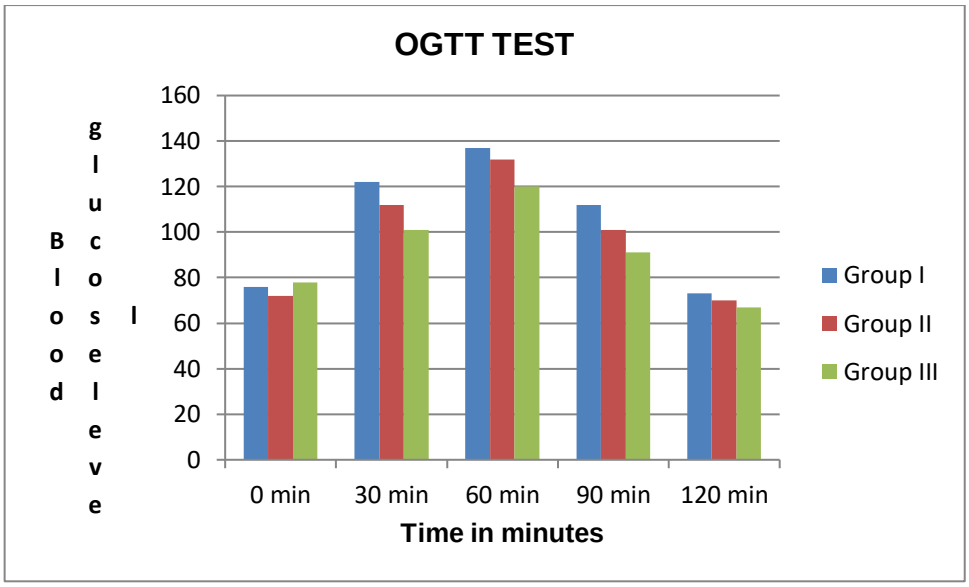


**Figure 21: Oral glucose tolerance test**

### 6.4.2. Effect of *Trigonella foenum graceum nanophytomes* and its aqueous extract on blood glucose level in experimental rats:

Single dose of streptozocin(65mg/kg) significantly increases the blood glucose as shown in table13 and figure 22. After daily administration with *Trigonella foenum graceum nanophytomes* (50 and 100 mg/kg p.o) and aqueous extract of *Trigonella foenum graceum* (200 mg/kg p.o) for 21 days, significant decreased in blood glucose levels was observed in diabetic rats. At the end of the experiment (21$^{st}$ day) blood glucose level was (145±2.25) mg/dl , (123.23±2.4) mg/dl and (148.25±2.33) mg/dl of the treated groups of doses of *Trigonella foenum graceum* nanophytosomes 50 and 100 mg/dl and aqueous extract of Trigonella foenum graceum 200mg/dl respectively.

**Table 13: represents blood glucose levels in rats**

| Groups | Blood glucose level (mg/dl) | | | |
|---|---|---|---|---|
| | Initial day | Day 7 | Day 14 | Day 21 |
| Group 1 | 115.27±4.50 | 113.34±3.80 | 112.7±5.20 | 113.82±2.40 |
| Group 2 | 253.52±2.45 | 296.54±4.35 | 325.36±4.27 | 389.24±4.34 |
| Group 3 | 255.24±2.28 | 201.23±3.52 | 130.31±2.34*** | 116.42±2.80*** |
| Group 4 | 252.21±2.24 | 234.20±2.25** | 197.20±2.25** | 145.22±2.25** |
| Group 5 | 265.52±2.70 | 226.43±2.90** | 158.58±4.30** | 123.23±2.41*** |
| Group 6 | 258.23±1.53 | 236.18±2.10*** | 199.24±3.23*** | 148.25±2.33** |

Values are expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. The values are ****P<0.0001, ***P<0.0005, **P<0.001 when compared against control.

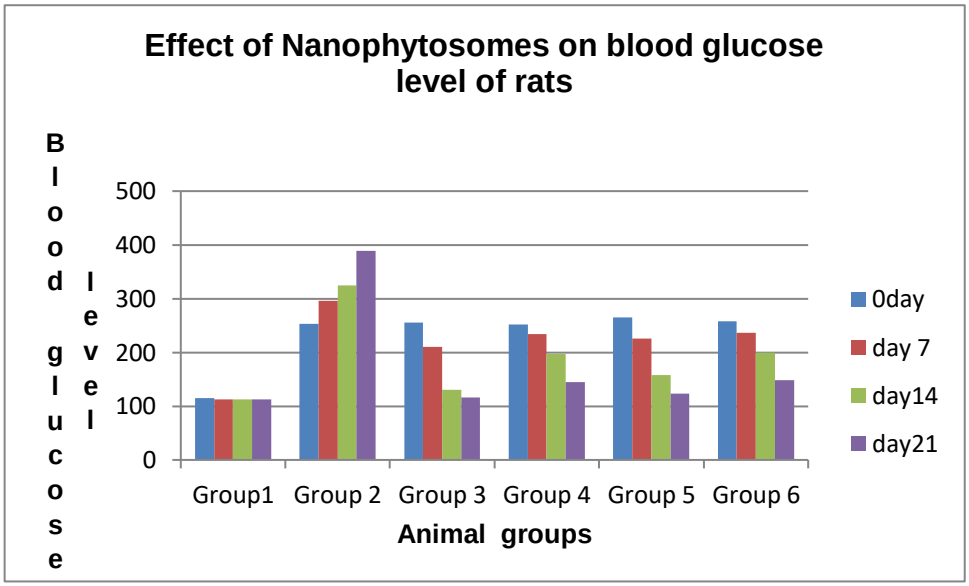


**Figure 22 Blood glucose level**

### 6.4.3. Effect of *Trigonella foenum graceum nanophytomes* and its aqueous extract on body weight in experimental rats:

In diabetic rats,continuous reduction of body weight was observed as shown in table14 and figure 23 .Metformin(250mg/kg) as well as the nanophytosomes(50 and 100 mg/kg) and aqueous extracts treatment significantly improved the body weight of diabetic rats.

**Table 14: represents body weight in rats**

| Groups | Body weight (g) | | | |
|---|---|---|---|---|
| | Initial day | Day 7 | Day 14 | Day 21 |
| Group 1 | 215.20±2.35 | 222.43±4.22 | 225.41±3.62 | 228.47±3.17 |
| Group 2 | 224.34±2.70 | 220.21±2.42 | 212.25±1.52 | 207.13±2.48 |
| Group 3 | 222.23±2.71 | 223.22±2.25 | 225.53±2.54** | 227.32±2.43** |
| Group 4 | 226.34±2.32 | 224.42±2.20** | 225.44±3.42 | 227.23±2.35** |
| Group 5 | 225.24±2.23 | 224.35±2.32 | 225.27±1.35** | 226.23±1.41*** |
| Group 6 | 228.23±1.53 | 226.18±2.10** | 227.24±3.23** | 228.25±2.33** |

Values are expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. The values are ****P<0.0001, ***P<0.0005, **P<0.001 when compared against control.

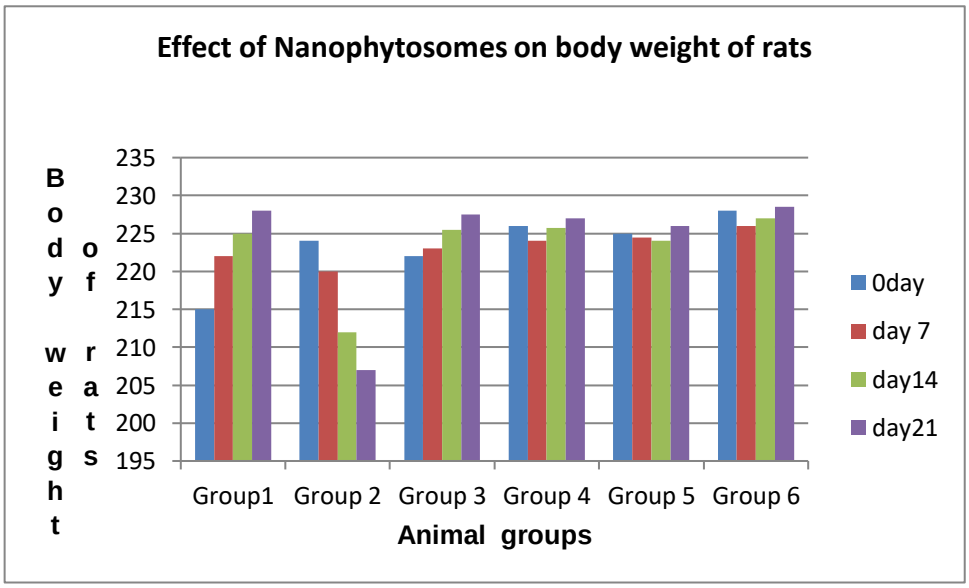


**Figure 23: Body weight of rats**

## 6.5. *INVITRO* ANTI-DIABETIC ACTIVITY:

### 6.5.1. *In Vitro* α-Amylase Inhibition Study

The maximum inhibition of *Trigonella foenum nanophytosomes* was 85.50% at a concentration 250 µg/mL. The percentage inhibition ranged from 85.50% - 30.83 %. The IC50 values are tabulated.

**Table 15: represents *invitro* antidiabetic activity by Alpha amylase inhibition method**

| Sample | Concentration(µg) | % Inhibition | $IC_{50}$ |
|---|---|---|---|
| Trigonella foenum graceum nanophytosomes | 50 | 30.83±0.6 | 140.49±0.33 |
| | 100 | 42.0±0.1 | |
| | 150 | 51.1±0.2 | |
| | 200 | 63.8±0.1 | |
| | 250 | 85.50±0.2 | |

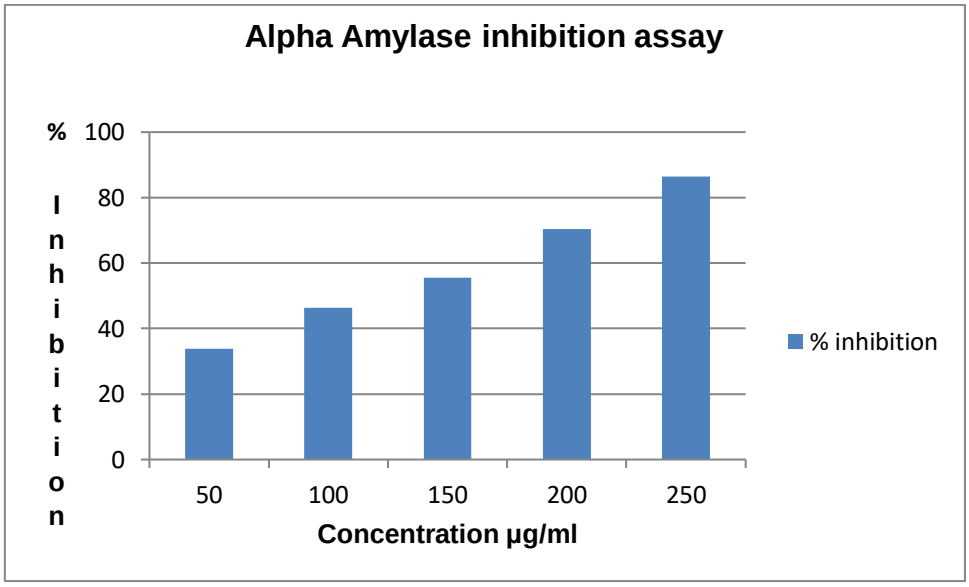


**Figure 24 reprents Alpha amylase inhibitory assay**

### 6.5.2.In *Vitro* α-glucosidase Inhibition Study

The in *vitro* α-glucosidase inhibitory studies demonstrated that *Trigonella foenum graceum* had α-glucosidase inhibitory activity. The highest concentration 250 µg/ml

tests showed a maximum inhibition of nearly 86.52 %. The percentage inhibition varied from 86.52 - 33.89 % from the highest concentration to the lowest concentration of 50 µg /ml. The IC50 values are tabulated.

**Table 16: represents invitro antidiabetic activity by Alpha glucosidase inhibition method**

| Sample | Concentration(µg) | % Inhibition | $IC_{50}$ |
|---|---|---|---|
| Trigonella foenum graceum nanophytosomes | 50 | 33.89±0.4 | 128.33±0.12 |
| | 100 | 46.33±0.0 | |
| | 150 | 55.61±0.4 | |
| | 200 | 70.48±0.2 | |
| | 250 | 86.52±0.4 | |

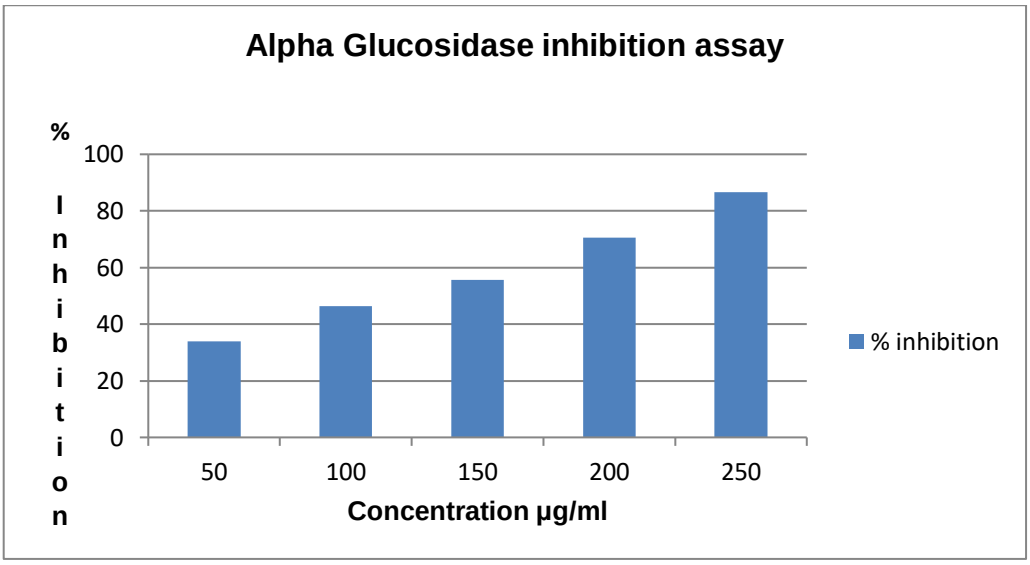


**Figure 25 represents Alpha glucosidase inhibition assay**

## 6.6. BIOCHEMICAL ESTIMATIONS

### 6.6.1. Estimation of Superoxide dismutase activity (SOD)

The *In vivo* antioxidant assay showed that the *Trigonella foenum graceum* nano-phytosome and extract increased the activity of serum superoxide dismutase (SOD). The SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitrite. The serum Superoxide dismutase activity of the group treated

with 50mg/kg of *Trigonella foenum graceum* nano-phytosome was significantly higher when compared to other treatment groups and the negative control group. The increased serum activity of SOD as observed in this study suggest that the *Trigonella foenum graceum* nano-phytosome and extract has an *In vivo* anti-oxidant activity and is capable of ameliorating the effect of ROS in biologic system (Table 17).

### 6.6.2. Estimation of Catalase activity (CAT)

Catalase is a ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production. The serum Catalase activity of the group treated with 50mg/kg of *Trigonella foenum graceum* nano-phytosome was significantly higher when compared to other treatment groups and the negative control group. The increased serum activities of catalase as observed in this study suggest that the *Trigonella foenum graceum* nano-phytosome and extract has an *Invivo* anti-oxidant activity and is capable of ameliorating the effect of ROS in biologic system. *Trigonella foenum graceum* nano-phytosome and extract caused a significant increase in the levels of bio-markers i.e., increased in the level of Catalase in a dose dependent manner.

### 6.6.3. Estimation of reduced glutathione (GSH)

GSH is a major intracellular non protein sulphydral compound and is accepted as the most important intracellular hydrophilic antioxidant. Also, GSH acts as a co-substrate for GPx activity and as a co-factor for many enzymes, stress resistance of many cells is associated with high intracellular levels of GSH. A decreased GSH content may predispose the cells to lower defense against condition of oxidative stress during several degenerative disease conditions. *Trigonella foenum graceum* nano-phytosome and extract caused a significant decrease in the levels reduced glutathione when compared to the control group but the serum reduced glutathione activity of the group treated with 50mg/kg of *Trigonella foenum graceum* nano-phytosome was significantly higher when compared to other treatment groups and the negative control group in a dose dependent manner.

### 6.6.4. Estimation of Thio-barbituric acid reactive substance(TBARS)

The *Invivo* antioxidant assay showed that the *Trigonella foenum graceum* nano-phytosome and extract increased the activity of TBARS. The serum TBARS activity of the group treated with 50mg/kg of *Trigonella foenum graceum* nano-phytosome was significantly higher when compared to other treatment groups and the negative control group (Table 17). The increased serum activity of TBARS as observed in this study suggest that the *Trigonella foenum graceum* nano-phytosome and extract has an *Invivo* anti-oxidant activity and is capable of ameliorating the effect of ROS in biologic system.

Some of the phytochemical constituents of the extract may be responsible for the anti-oxidant activity as demonstrated in our study. Flavonoids or bioflavonoids are a ubiquitous group of polyphenolic substances which are present in most plants, concentrated in seeds, fruit skin or peel, bark, and flowers. Numerous studies have shown that flavonoids possess potent antioxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals.

**Table17 Bio-marker changes**

| Treatment | SOD (units/ml) | CAT(µmol/mg protein) | GSH(µmol/mg protein) | TBARS (nmol/mg of Protein) |
|---|---|---|---|---|
| **Group 1** | 11.31±0.13 | 45.19 ±0.13 | 83.22±0.23 | 2.49±0.21 |
| **Group 2** | 9.18±0.14*** | 32.72± 0.29**** | 56.53±0.24**** | 1.53±0.24** |
| **Group 3** | 16.24±0.17**** | 54.37±0.31**** | 43.36±0.29**** | 4.39±0.13**** |
| **Group 4** | 18.36±0.22**** | 78.72±0.29**** | 73.24±0.17**** | 4.89±0.04**** |
| **Group 5** | 21.41±0.43**** | 104.32±0.28**** | 79.61±0.29**** | 5.62±0.05**** |
| **Group 6** | 17.15±0.28**** | 81.41±0.30**** | 60.19±0.49**** | 4.38±0.01**** |

Values are expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. The values are ****P<0.0001, ***P<0.0005, **P<0.001 when compared against control.

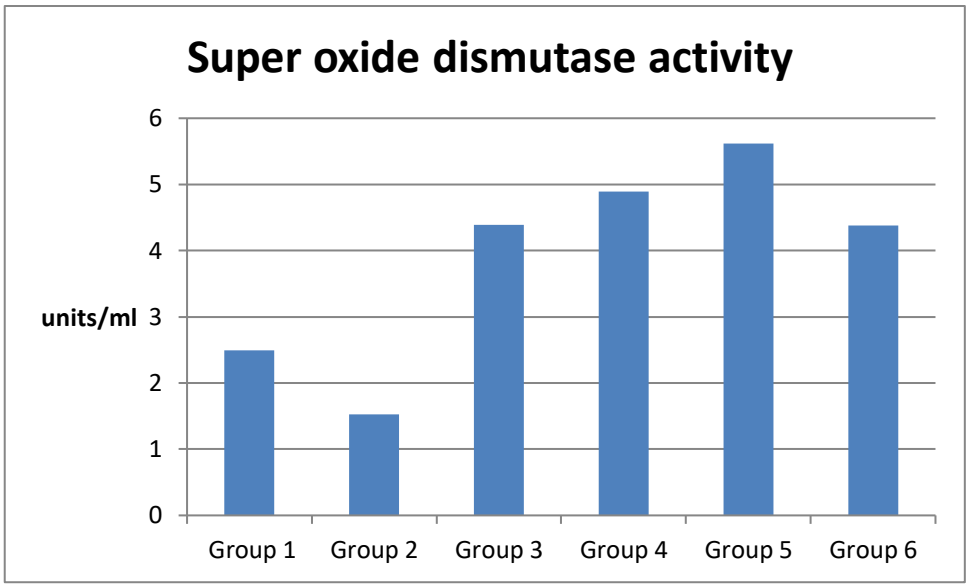


**Figure 26 Estimation of Superoxide dismutase activity (SOD)**

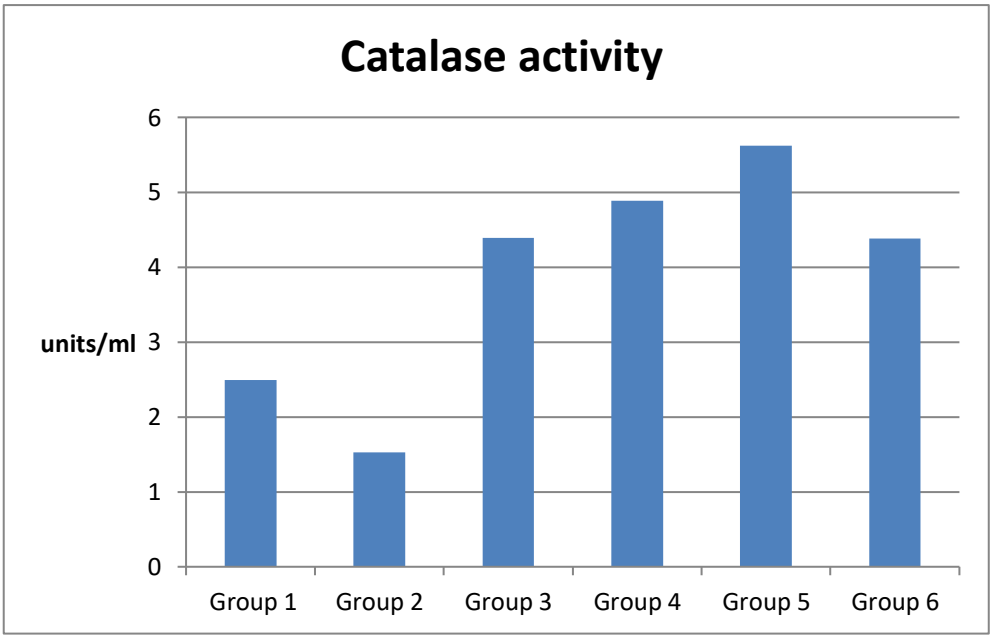


**Figure 27 Estimation of Catalase activity**

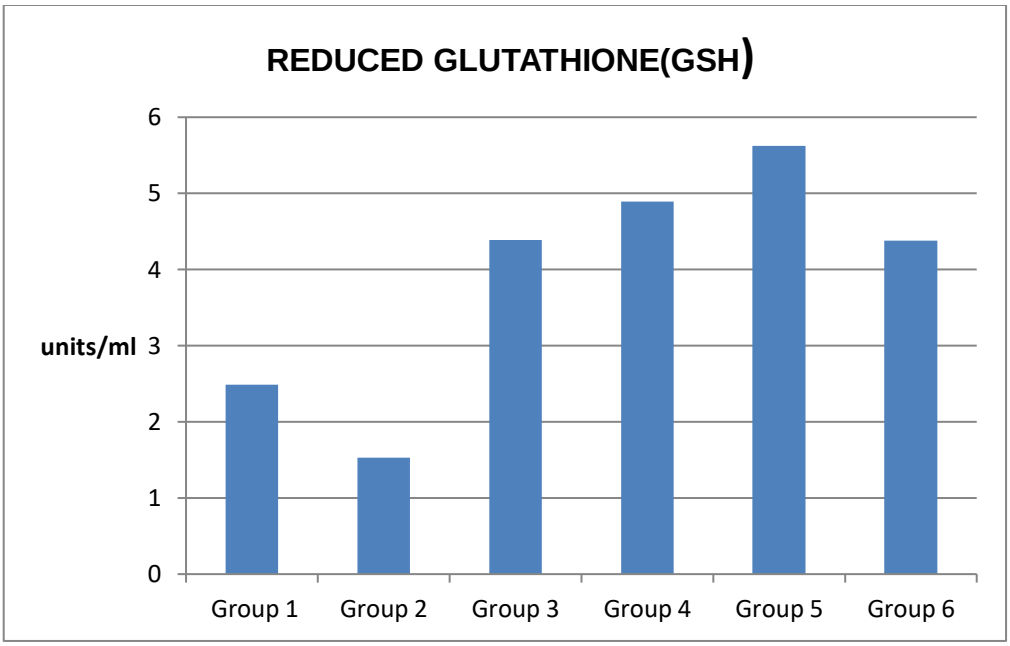


**Figure 28 Estimation of Reduced Glutathione (GSH)**

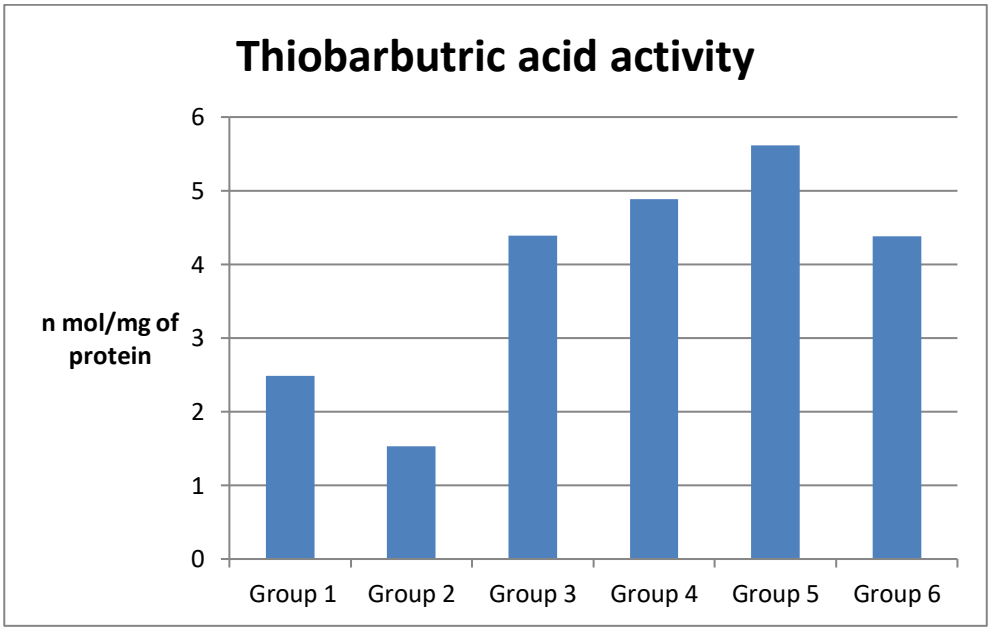


**Figure 29 Estimation of TBARS**

## 6.7. HISTOPATHOLOGICAL STUDIES

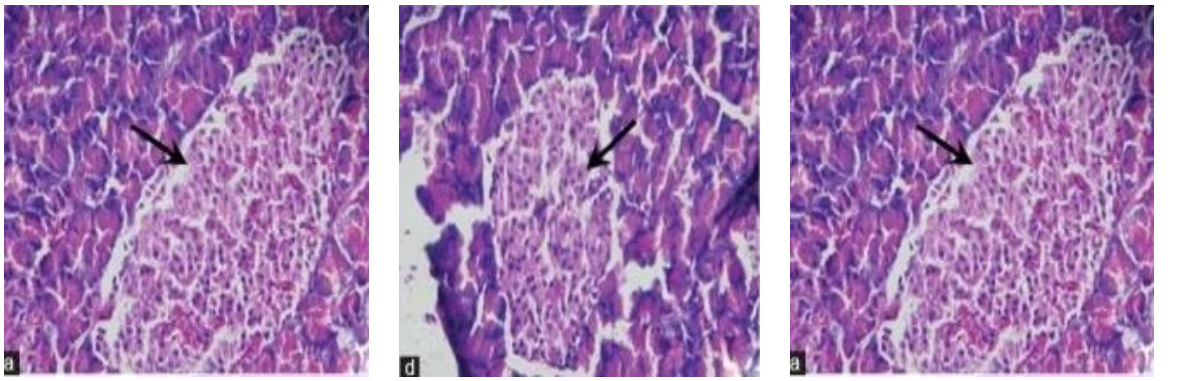

**Figure a** **Figure b** **Figure c**

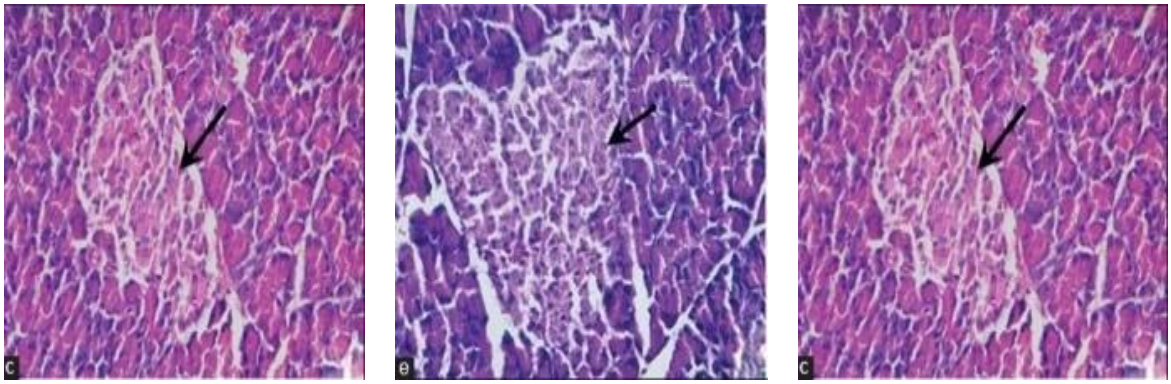

**Figure d** **Figure e** **Figure f**

**Figure 30 Histological features of Rat pancreas**

Histology of pancreas (Figure 30) showed normal acini, and normal cellular in the islets of langerhans in the pancreas of normal control (Figure 30 a). In diabetic animals treated extensivedamage to islets of langerhans and reduced dimensions of islets were observed in diabetic rats (Figure 30 b) which were restorated toward normal cellular population size of islets by *Tigonella foenum graceum* nanophytosomes (50mg/kg and 100mg/kg)(Figure 30 d,e) and *Trigonella foenum graceum* extract (200mg/kg)(Figure 30 f) and metformin treated groups(Figure 30 c).

# CHAPTER – VII

# CONCLUSION

# CHAPTER VII

# CONCLUSION

It was clearly understood that, the plant *Trigonella foenum graceum* is having many phyto constituents like Phenols, flavonols, proanthocyanidins and flavonoids which are very important plant bioactive components reported to possess strong anti-oxidant and antidiabetic activities.

*Trigonella foenum graceum* nano-phytosome complex was successfully formed by thin layer lipid hydration method with sufficient vesicle size, vesicle size distribution. Characterization was done by SEM, TEM, DSC, FT-IR spectroscopy, Particle size and Zeta potential confirmed that hydrogen bonds or van der Waals force contributed in the *Trigonella foenum graceum* extract and phospholipids complexation. From the above studies it was concluded that phytosomes has better physical characteristics than that of extract.

*In-vitro* studies revealed that *Trigonella foenum graceum* nano-phytosomes have better anti-oxidant, anti-diabetic activity when compared with aqueous extract of leaves of *Trigonella foenum graceum.*

Diabetes mellitus is a most common endocrine disorder, which affects the people worldwide. To control this disorder many allopathic drugs are available .Those drugs are often having severe adverse effect, unpredictable cost which pave the way for the use of herbal medicines in the treatment of diabetes. In the present study single peritoneal injection of Streptozocin induce diabetes within 2-3days.The symptoms like elevated in blood glucose level and reduction in body weight gradually improves during the treatment of 21 days orally with *Trigonella foenum graceum* nanophytosomes and extract.In this study, we observed that Trigonella foenum graceum nanophytosomes significantly improves the conditions of diabetics when compared to *Trigonella foenum graceum* extract. It is concluded that, the anti-diabetic activity of Trigonella foenum graceum has enhanced by formulating their nanophhtosomes.

# CHAPTER – VIII

# BIBLIOGRAPHY

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## BIBLIOGRAPHY


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Review Article

# A REVIEW ON PHYTOSOMES, IMPORTANCE AND ITS APPLICATIONS

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**ABSTRACT**

Phytosomes are said to be natural extracts contains plant constituents which are bound in phospholipids mainly phosphotidylcholine by producing a lipid stable molecular complexes. This will become better formulation with high grade of stability to attain peak pharmacokinetic and pharmacodynamic profiles. Such degree of freedom will pave to chart out many therapeutic interventions towards the treatment of most of diseases. Hence, The authors has taken a lead to emphasize the importance of phytosomes, its preparations and characterization and also current scenario towards phytosomal technology in detailed way.

**Key words:** Nanotechnology; Plant products; Nanophytosomes.

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## INTRODUCTION

Phytosomes are said to be containing natural herbal formulations. Most of the Plants are having medicinal properties due to the presence of many active constituents which are mainly the secondary metabolites like flavonoids, terpinoids, tannins, glycosides, alkaloids etc. The active constituents present in the plants are mostly hydrophilic in nature. The therapeutic efficacy of herbal extracts are quickly destroyed by the enzymes present in the intestinal gut. Hence, advanced researches are done for the specific site delivery of these plants derived products (Middleton and Kandaswami,1994).The term “phyto” means plant and “some” means cell like (Mukherjee and Wahile,2006). It is also called as herbosomes. This is an advanced methodology, where extract of the plant or the hydrophilic phytoconstituents are mixed with phospholipids to produce more lipid stable molecular complexes, thereby it enhances the absorption and bioavailability of phytoconstituents (Manach *et al*,, 2004; Mascarell, 1993). Phospholipids are naturally used as an aid for digestion and act as carriers for both fat soluble and water soluble nutrients (Shivan and Kinjal, 2010). Phytosomes can easily cross the cell membranes and also stratum corneum layer of the skin (Bombardelli *et al*., 1989; Bombardelli, 1991; Bombardelli and Spelta,

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1991; Loggia *et al.,* 1996; Forster *et al*., 2009; Chanchal and Swarnlata, 2008). In the last century numerous research have been performed on a lot of plant extracts to know their biological importance and their use in medicinal field (Middleton and Kandaswami,1994). Phytosomes have better ability to penetrate into the membrane of the cell and from there it enter into the cell and finally reaching the systemic circulation (Bombardelli *et al*.,1989).

**Phytosome Technology and Its Advantages**

Hydrophilic phytoconstituents has the ability to bind with phospholipids. A specified amount of phospholipid (phosphatidylcholine) react with the herbal extract in a non-polar solvent. The phospholipid (phosphatidylcholine) used in this formulation was obtained from soybean with both lipophilic (phosphoyidyl part) and hydroplilic (choline) portions. The body portion has choline group which is hydrophilic and the tail portion has phosphotidyl group which is lipophilic in nature, thereby the hydrophilic group is encoded within the lipophilic group to form a stable complex, phytosomes are formed (Goyal *et al.,* 2011; Keerthi *et al*., 2014).The bonds formed are chemical in nature, which in additional provides better stability for the drug molecule in complex with wide range of advantages (Table 1). Phosphatidylcholine used in this formulation has dual function, it act as a carrier for drug moiety with nutritional value (Amin and Bhat, 2012; Singh *et al.,* 2013).

**Preparation of Phytosomes**

Though there was not enough data available throughout the phytosome research, authors tried maximum to provide all inputs for the preparation of phytosomes. The method for the preparation of phytosomes are as follows: In the first step, phospholipids are obtained from either natural or synthetic sources are to be dissolved in a organic solvent such as acetone or dioxane. To the solution of phospholipids, herbal extract is added with constant stirring. Then the solution is allowed to evaporate on a spray dryer. The ratio between the portions in the range of 0.5 to 2.0 moles but the most preferable ratio is1:1(Karimi *et al*., 2015; Rani *et al.,* 2007). Thin flim is formed after evaporation of the solvent. Futher hydration of the flim leads to formation of phytosomal suspension. The formed phytosomes will be collected by precipitation technique. The collected phytosomes are futher subjected to drying by lyophilisation method (Pandey and Patel,2010; Saha *et al.,* 2013). The entire preparations are illustrated in schematic way for better understanding to the users (Fig 2).

**Characterization and Evaluation of Phytosomes**

The characterization techniques for the evaluation of phytosomes are as follows (Patel *et al.,* 2013)

Characterization techniques:

**Vesicle size and Zeta potential:** The particle size and zeta potential can be determined by DLS using a computerized inspection system (Patel *et al.,* 2004).

**Surface morphology analysis:** By using scanning electron microscopy (SEM) the surface morphology analysis of phytosomes can be determined (Tripathy *et al.,* 2013).

**Transition temperature:** By using differential scanning calorimetry the transition temperature of the vesicular lipid system can be determined.

**Surface tension measurement**: Du novy ring densitometer is used to find out the surface tension activity of a drug dissolved in aqueous solution (Pawar and Bhangale, 2015).

**Entrapment efficiency**: By using ultracentrifugation technique the drug entrapment ability of phytosomes can be measured (Patel *et al.,* 2004).

**Drug content**: Drug content present in the phytosomes can be determined by High performance liquid chromatographic method or any other spectroscopic methods (Bhattacharya, 2009).

**Stability studies:** Stability studies were carried out for two months on the optimized formulation of phytosomes. For stability study the optimized formulation were placed in humidity chamber (sonar) at 75%RH,$45^0$c. After two months, the formulation was evaluated for weight variation, hardness, friability, disintegration and percentage drug content (Kumari *et al.,* 2011).

**SPECTROSCOPIC EVALUATIONS**

The spectroscopic evaluation provides more information about phytosomes. They are as follows:

**FT-IR:**The FT-IR spectra data will be taken to determine the structure and chemical shift of the extract, phosphotidylcholine and phytosomes (Pawar and Bhangale, 2015).

**$^1$H-NMR:** The $^1$H-NMRspectra is used to determine the development of complex formed between active phytoconstituents and phosphotidylcholine molecules. In non polar solvents, there will be an evident change in $^1$H-NMR signal commencing from atoms included in the complex formation. The signals from protons are broadened. In phospholipids there is broadening of signals whereas the singlet correlative to the N-trimethyl portion of choline yields an upfield shift (Tripathy *et al.,* 2013).

**$^{13}$C-NMR:** The $^{13}$C-NMR of phytosomes, when recorded at room temperature all the carbons in phytoconstituents are unobservable. The signals equivalent to the choline and glycerol portion was broadened, whereas some are shifted and most of the resonance of the fatty acids chains maintains their initial sharp lines (Gupta and Dixit, 2011).

**Current Research Towards Nanophytosomes**

Since the phytosomes are novel in upcoming era, few extensive studies are done on silymarin, grape seed extract, quercetin, curcumin etc (Wellington and Jarvis, 2001; Hikino *et al*,1984).Some are enlisted have for the reading: In *Schandalik et al.*, 1992, 1994, has used nine human volunteer patients and tested the hepatoprotective activity of silymarin and reported that phytosomal form of silybin possess four times greater passage through the liver (Schandalik and Perucca,1994; Schandalik *et al.*,1992). In 1993, *Mascarella et al*, conducted similar work by using 232 patients with chronic hepatitis and reported the better bioavailability of silymarin phytosome[La Grange *et al.*,1999]. Grape seed phytosome is composed of oligomericpolyphenols, proanthocyanidins or procyanidins (*Vitisvinifera*) of varying molecular size, complexed with phospholipids. The main properties of procyanidin flavonoids are with increased antioxidant capacity and stimulation of physiological antioxidant defenses of plasma, protection against ischemia/reperfusion induced damages in the heart, protective effects against atherosclerosis thereby offering marked protection for the cardiovascular system and other organs through a network of mechanisms that extend beyond their great antioxidant potency. In the year of 2001, *Jhiang* prepared herb epimedii flavanoidphytosome (EPF). The precipitate was investigated and the study showed that the dissolution of the precipitate was significantly higher than that of its physical mixture and Herba epimedii extract tablets (Jiang *et al.*, 2001). In 2005*Bombardelliet al*, reported that the silymarin phytosomes showed much site specific activity and a longer duration of action than the single constituent, with respect to percent reduction of edema, inhibition of myeloperoxidase activity, antioxidant and free radical scavenging activity (Bombardelli *et al*., 1991). In 2005*Maitiet al* produced a quercetin phytosome by a simple and reproducible method and reported that the phytosomal complex shows better therapeutic effect than the uncomplexed molecule in rat liver injury induced by carbon tetrachloride (Jiang *et al.*, 2001; Maiti *et al.*, 2005). Maiti *et al*, 2006, 2007, also developed naringenin and curcumin phytosomes in two different studies and reported that antioxidant activity of the phytosomal complex has better therapeutic efficacy than that of normal one (Maiti *et al.*, 2010). In *xiao et al.*, 2006, prepared silymarin phytosomes and studied its pharmacokinetics in rats and reported that the bioavailability of silybin has increased markedly with phytosomal formulations. Hepatoprotective activity of silymarin phytosomes was found to be more than silymarin alone against aflatoxin B1, as reported by *Tedescoet al*, 2008, after performing the experiment on broiler chicks (Tedesco *et al.*, 2004). Hence, In recent years much works are going to focus on standardized herbal extracts to formulate into more bioavailable phytosomes. Extract of *Serenoa repens*(CO2 extract) extract of *Vaccinium myrtillus* (Fruit extract), extract of *Coleus forskohlii*, Ximenoil and Ximenynic acid extracted from *Santalum album*, Esculoside, glycosylated coumarin obtained from *Aesculus hippocastanum*, Ruscogenins, group of saponins extracted from *Ruscus aculeatus* are highly worked upon for better bioavailability through the formation of phytosomes by patented process (Acharya *et al.*, 2011).

**Commercial Products in Market**

To date, very few products has come in to market and said to be commercially available. The list of available (Table 2) components is enlisted here for readers.

**Table 1. Advantages of Phytosomes**

| SNO | **Advantages of Phytosomes (**Kidd and Head, 2005; Semalty *et al.*, 2007; Naik and Panda, 2008; Bhattacharya,2009). |
|---|---|
| 1. | Hydrophilic herbal extracts absorption is enhanced and has a better therapeutic effect. |
| 2. | Phytosomes deliver the drug at specific site, so low dose is required to produce therapeutic effect. |
| 3. | Phytosomes are easy to develop and has more stability than any other herbal formulations. |
| 4. | The carrier used in this formulation (phosphotidylcholine)has an advantage that it is eco-friendly with nutritional value. |
| 5. | Drug entrapment capacity of phytosomes is high than compared to any other herbal formulations. |
| 6. | No complex techniques are not required in the production of phytosomes and hence low cost is required for its production. |
| 7. | Phytosomal formulations are easily penetrate through the layer of the skin. Hence it can use for transdermal delivery. |

**Table 2. Commercial products of phytosomes**

| S.No | Phytosomes | Phytoconstituents | Therapeutic Applications |
|---|---|---|---|
| 1. | Silybin Phytosome | Silybin from Silybummarianum | Hepatoprotective, antioxidant for liver and skin |
| 2. | Ginkgo Phytosome | 24% ginkgo flavonoids | Protects brain and vascular linings, anti-skin Ageing |
| 3. | Ginseng Phytosome | 37.5% ginsenosides | Nutraceuticals, immunomodulator |
| 4. | Green Tea Phytosome | Epigallocatechin | Nutraceutical, systemic antioxidant, anticancer |
| 5. | Grape Seed Phytosome | Procyanidins | Nutraceutical, systemic antioxidant, cardioprotective |
| 6. | Hawthorn Phytosome | Flavonoids | Nutraceutical, cardio-protective and antihypertensive. |
| 7. | Olive oil Phytosome | Polyphenols | Antioxidant, anti-inflammatory, antihyperlipidemic |
| 8. | Echinacea Phytosome | Echinacosides | Nutraceutical, immunomodulator |
| 9. | Centella Phytosome | Terpenes | Vein and Skin disorders |
| 10. | Palmetto berries Phytosomes | Fatty acids, alcohols and sterols | Non-cancerous prostate enlargement |

**Fig 1(A & B). The difference between phytosome and liposome.**

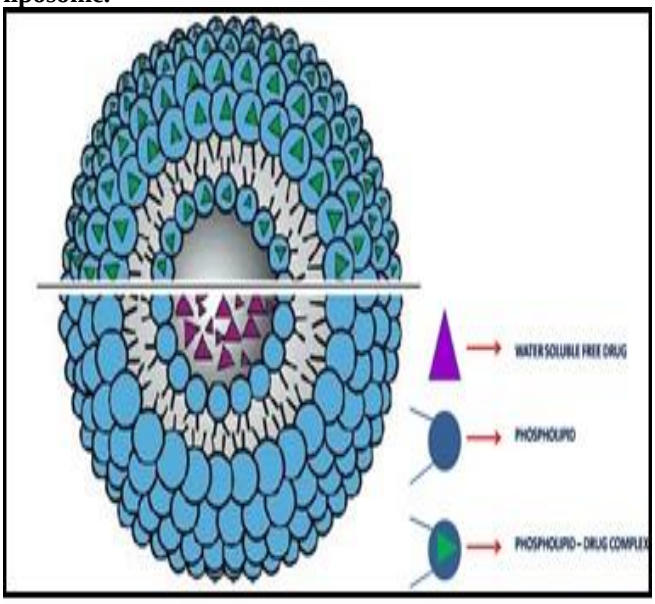



**Fig 2. Schematic illustration of preparation of phytosomes.**

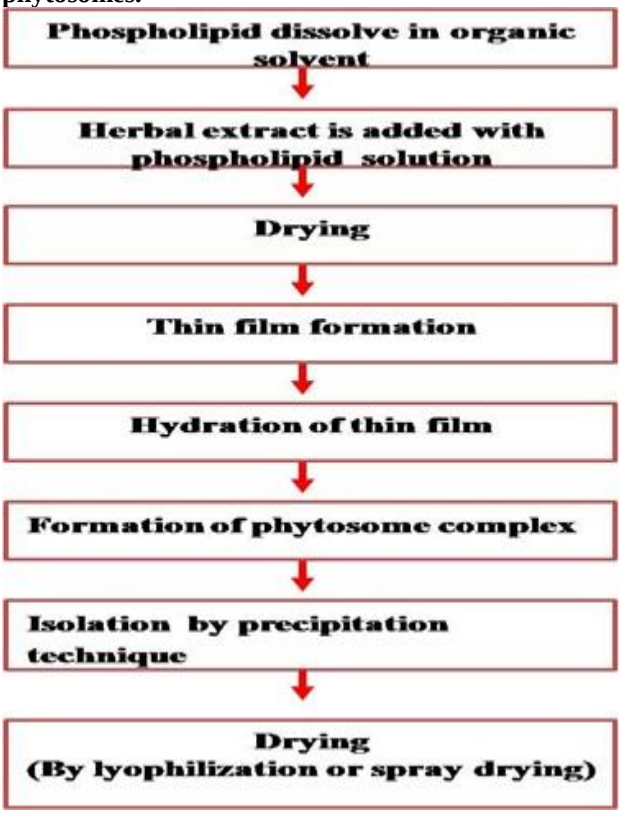
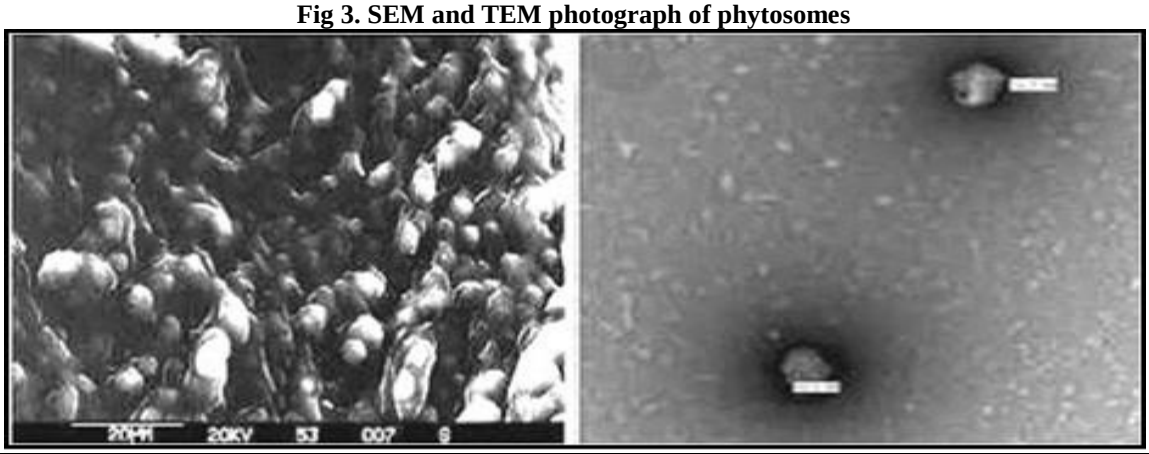


**Fig 3. SEM and TEM photograph of phytosomes**



## CONCLUSION

Phytosomes or herbosomes are said to be advances in herbal formulations. Such phytosomal technology forms a strong link among conventional and novel drug delivery systems. Apart, phytosomes will have better pharmacokinetic and pharmacological efficacy. It also has better therapeutic effect at low dose to produce desired pharmacological effect. Phytosomes have wide scope in nutraceuticals and cosmetology. Hence in future, nanophytosomes will play an important role in the field of drug delivery with high peak values at affordable cost to treat many chronic and acute diseases.

## CONFLICTS OF INTEREST

Authors declare no conflicts**.**

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